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(54) Title: DETECTION OF CHROMOSOMAL ABNORMALITIES

(57) Abstract

The present invention provides a method for detection of the presence or absence of chromosomal abnormalities which are associated with a condition in a subject and are each defined by at least one characteristic nucleic acid sequence. In general, the method comprises subjecting a sample of nucleic acids to a multiplex molecular amplification procedure. The multiplex molecular amplification procedure comprises the use of at least 7 mutually distinct primers in one single reaction mixture, each of the at least 7 mutually distinct primers defining an end of at least one characteristic nucleic acid sequence, and wherein at least one of the at least 7 mutually distinct primers defines the first ends of at least two characteristic nucleic acid sequences, said at least two characteristic nucleic acid sequences being defined in their opposite ends by mutually distinct primers selected from the remainder of the at least 7 mutually distinct primers, whereby the number of amplified characteristic nucleic acid sequences which can be detected upon conclusion of the amplification reaction is at least 1/2xn+1, wherein n is the number of the at least 7 mutually distinct primers. In one embodiment, the use of an internal positive standard containing: I) a nucleic acid fragment present in the sample, and II) primers for amplification of a nucleotide sequence of said nucleic acid fragment is incorporated into the procedure.

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DETECTION OF CHROMOSOMAL ABNORMALITIES

FIELD OF THE INVENTION

The present invention relates to methods for detection of the presence or absence of chromosomal abnormalities associated with a condition, notably a malignant neoplastic disease, in a subject and defined by at least one characteristic nucleic acid sequence. The invention further relates to DNA fragments having specific nucleic acid sequences and their use as either cDNA primers or primers in molecular amplification reactions leading to the detection of chromosomal abnormalities. The invention also relates to a kit comprising selected primers for use of detection according to the invention.

GENERAL BACKGROUND

Chromosomal translocations appear to be important events in the development of tumours (especially haematopoietic tumours) and more than 50 different consistently occurring translocations have been described (Rabbitts, T. H. Nature 372:143 (1994)). Many of the chromosomal aberrations have been found to be specific to particular subtypes of leukaemia or lymphoma.

The identification of translocations in haematopoietic malignancies is therefore of great diagnostic and prognostic value. The diagnosis of acute leukaemia is multidisciplinary with standard pathology, immunology and cytogenetics as the most often used methodologies. In this setting, immunophenotyping using flow cytometry and monoclonal antibodies provide a speedy and accurate differentiation between lymphoid and myeloid lineages, while a bone marrow biopsy delineates the degree of malignant infiltration simultaneously with revealing the extent of remaining normal haematopoiesis. Neither immunophenotyping nor histology seem to be able to provide satisfactory tools for prognosticating the patients. In

contrast, cytogenetic evaluation, while being time consuming, has been shown to delineate both patient groups with favourable as well as poor prognosis. The basis for the value of cytogenetics as a prognostic tool is the existence of a number of balanced chromosomal translocations, where unique genetic sequences are created (for review see Rabbitts, 1995). Cloning of the translocation break points have indicated that these genes can be altered at the level of their expression or in the properties of the encoded proteins. These alterations appear to play an integral role in the development and possibly in the progression of the disease.

Molecular studies of chromosomal rearrangements connected with the development of haematopoietic tumours have provided important insights into the mechanism of tumorigenesis. The translocations may alter the function or activities of cellular proto-oncogenes located at or near the breakpoint. These proto-oncogenes are normally involved in control of cellular growth, differentiation or apoptosis. The oncogenic conversion may occur by two general mechanisms, either (i) by juxtaposition of a cellular proto-oncogene to the regulatory element of a tissue specific gene, e.g. the immunoglobulin and T-cell receptor genes in leukaemia, leading to inappropriate expression of the oncogene (Leder, P. et al., Science 222:765 (1983); Finger, L. R. et al., Science 234:982 (1986)), or (ii) by creating fusion genes coding for chimeric proteins with functional features different from the wild-type protein (Borrow, A. D. et al., Science 249:1577 (1990); de Thé, H. et al., Nature 347:558 (1990)).

Translocational breakpoints are highly conserved and generally within the introns of the affected genes. This is properly due to constrains on the reading frame and on protein (mal)function, but also intron size and the presence of repeated (e.g. Alu) sequences or sequences homologous to B- and T-cell specific recombinase recognition sites within the introns may target and influence the frequency of translocations. However a number of fusion-genes have been found in

several variant sizes. Sequence analysis has revealed that the reading frame of the fusion protein variants is preserved, and shows that there may be some freedom in the joining of protein domains in the generation of the oncogene.

In both acute myeloid and lymphoid leukemias, one of the genes involved in the fusion is most often a transcription factor which appears to have a direct role in haematopoiesis and which, following the translocation, is frequently fused to a second gene not normally active in haematopoietic cells. In some instances the same gene is involved in fusion with more that one chromosomal partner.

A translocational breakpoint gene may have several fusion partners, the most promiscuous example is the MLL gene at chromosome band 11q23, where 10 different fusion partners together with an internal duplication has been described. The MLL/AF4 fusion gene, detected in t(4;11)(q21;q23) translocations, is only observed in paediatric ALL, whereas the MLL/AF6 fusion gene detected in t(6;11)(q27;q23) translocations is seen in a subgroup of AML patients (Prasad, R, et al., Cancer Res. 53:5624 (1993)). The t(10;11)(p14;q23) translocation, where the MLL is fused to the AF10 gene, has been described in both paediatric ALL and AML patients. Thus depending on fusion partner the MLL gene can contribute to the pathogenesis of either lymphoid or myeloid malignancies or both. A number of breakpoint genes have been found fused to various partners in different translocations. e.g. E2A/PBX and E2A/HLF in t(1;19)(q23;p13) and t(17;19)(q22;p13) or PML/RARα, PLZF/RARα, NPM/RARα, NPM/ALK, NPM/MLF in t(15;17)(q22;q21), t(11;17)(q23;q21), t(5;17)(q35;q21), t(2;5)(p23;q35), t(3;5)(q25.1;q35), respectively. Thus at least a subset of the translocations detected in haematopoietic malignancies can be grouped into "fusion-gene families".

Identification of translocations has generally been performed with karyotyping by G-banding or more recently by Fluorescent In Situ Hybridization (FISH). However, chromosome preparation

from clinical samples is often not feasible and the cytogenetic based diagnoses are not sensitive to a small fraction of abnormal cell, i.e. are not helpful for monitoring for relapse. Cytogenetic analysis may detect gross aberrations, but not submicroscopic alterations. Some of these problems may be overcome by using PCR based techniques. However, only four different chromosomal abnormalities have been identified by multiplex-PCR, Repp, R. et al; Detection of Four Different 11q23 Chromosomal Abnormalities by Multiplex-PCR and Flourescence-Based Automatic DNA-Fragment Analysis:Leukemia(1995)9:210-215

In 10-30% of the patients with a normal karyotype a translocation specific fusion gene can be detected by PCR techniques, indicating that a second chromosomal rearrangement has occurred restoring the normal karyotype. A PCR analysis is rapid and very sensitive, but will generally only detect one specific fusion-gene. A major drawback of the PCR method is that it is time consuming and that false negatives are difficult to detect. Thus, the high number of diversified translocations, which have hitherto been demonstrated in acute leukaemia, has precluded its use as a screening tool.

For nearly all translocations, where the genes involved have been identified, a PCR based technique for the detection of the fusion-gene has been described. However, due to the variation in reaction conditions and detection systems, the number of fusion-genes, and the amount of patient material needed, it would be almost impossible with the present methods to screen a patient for the fusion-genes described.

Thus, there is a definite need for fast and reliable screening methods which render possible a prognostic evaluation of patients suspected of suffering from e.g. malignant diseases.

OBJECT OF THE INVENTION

It is an object of the invention to provide a simplified and reliable method for the detection of families of chromosomal abnormalities (such as translocations), which do not suffer the drawback of the prior art methods. Accordingly, a fast and safe method resulting in a very specific diagnosis, it is a futher advantage that the method may be performed by use of as small amounts of sample as possible, due the fact that the patients includes children and babies from which even 20 ml blood is a considerable amount. Further, it is an object of the invention to provide means (especially in the form of specific useful primers) for such a novel method.

DESCRIPTION OF THE INVENTION

The inventors of the present invention have provided a method for detection of the presence or absence of chromosomal abnormalities which are associated with a condition in a subject and are each defined by at least one characteristic nucleic acid sequence. In general, the method comprises subjecting a sample of nucleic acids to a multiplex molecular amplification procedure using multiple and mutually distinct primers in one single reaction mixture, wherein each of the primers defines an end of at least one characteristic nucleic acid sequence. The method according to the invention makes it possible, with a minor amount of work, to screen a sample of nucleic acids for a very high number of chromosomal abnormalities which may occur in a subject. By employing the methods of the invention it has become possible to detect about 50 chromosomal rearrangements with more than 80 subtypes, all performed in one single experimental round.

One distinguishing feature of the invention is the use of amplification primers which constitute the one half of more than one pair of primers used in the amplification reaction,

thereby reducing the number of primers necessary to carry out the amplification of all sequences of interest.

For instance, if combining the prior art methods, it would be necessary to use two primers for each individual chromosomal abnormality to be detected. The present invention, on the other hand, exploits the fact that e.g. translocations fall within families, wherein one half of one translocation product is present in at least one other translocation product. In the simple situation where two such translocation products should be detected, it would only be necessary to use a total of three primers. In a more complicated setup, where e.g. 5 translocation products (which all share one translocation "half") should be detected, a total number of 6 primers could ideally be used; this is in strong contrast to the 10 primers which would be necessary when performing a multiplex amplification utilizing the prior art primer systems.

Hence, the inventor has utilized the existence of families of translocations so as to reduce the total number of primers necessary to detect virtually all known chromosomal abnormalities associated with malignant diseases of haematopoietic origin. In addition, it has been achieved to perform multiplex PCR reactions wherein the number of primers is no less than 7, *i.e.* an unprecedented high number of primers present in a multiplex PCR for this purpose.

Thus, in a first aspect the invention relates to a method for detection of the presence or absence of chromosomal abnormalities, each of these chromosomal abnormalities being associated with a condition in a subject and each of these chromosomal abnormalities being defined by at least one characteristic nucleic acid sequence, the method comprising

a) obtaining a sample of nucleic acids derived from a subject which may harbour one of said chromosomal abnormalities,

b) subjecting the sample of nucleic acids to a multiplex molecular amplification procedure, wherein a number of said characteristic nucleic acid sequences, if present in a sufficient amount, will be amplified,

c) retrieving the product(s) from step b), and detecting the presence and/or absence of amplified characteristic nucleic acid sequences and thereby the presence or absence of corresponding chromosomal abnormalities,

wherein the multiplex molecular amplification procedure comprises the use of at least 7 mutually distinct primers in one single reaction mixture, each of the at least 7 mutually distinct primers defining an end of at least one characteristic nucleic acid sequence, and wherein at least one of the at least 7 mutually distinct primers defines the first ends of at least two characteristic nucleic acid sequences, said at least two characteristic nucleic acid sequences each being defined in their opposite ends by mutually distinct primers selected from the remainder of the at least 7 mutually distinct primers, whereby the number of amplified characteristic nucleic acid sequences which can be detected upon conclusion of the amplification reaction is at least ½xn+1, wherein n is the number of the at least 7 mutually distinct primers.

In order to obtain reliable results from the molecular amplification procedures used in the methods of the invention, it is often necessary to ensure that the molecular amplification has been satisfactorily performed, *i.e.* to avoid false negative readings upon conclusion of the amplification.

According to the invention, this can be done by amplifying an internal standard (in the form of a nucleic acid fragment) in the reaction mixture together with a set of primers which will initiate and sustain amplification of the standard.

Therefore, in another aspect, the invention pertains to a method for detection of the presence or absence of chromosomal abnormalities, each chromosomal abnormality being associated with a condition in a subject and each chromosomal abnormality being defined by at least one characteristic nucleic acid sequence, the method comprising

a) obtaining a sample of nucleic acids derived from a subject which may harbour one of said chromosomal abnormalities,

b) subjecting the sample of nucleic acids to a multiplex molecular amplification procedure, wherein a number of said characteristic nucleic acid sequences, if present in a sufficient amount, will be amplified,

c) retrieving the product(s) from step b), and detecting the presence and/or absence of amplified characteristic nucleic acid sequences and thereby the presence or absence of corresponding chromosomal abnormalities,

wherein the multiplex molecular amplification reaction comprises

1) the use of an internal positive standard containing I) a nucleic acid fragment present in the sample, and II) primers for amplification of a nucleotide sequence of said nucleic acid fragment, and

2) a number, n, of mutually distinct primers each defining an end of a characteristic nucleic acid sequence,

and wherein at least one of the n mutually distinct primers defines first ends of at least two mutually distinct characteristic nucleic acid sequences, said at least two mutually distinct characteristic nucleic acid sequences being defined in their opposite ends by at least two mutually distinct primers selected from the remainder of the n mutually dis-

tinct primers, whereby the number of amplified characteristic nucleic acid sequences which can be detected upon conclusion of the amplification procedure is at least ½×n+1.

When using the phrases "method of the invention" or "methods of the invention" is herein meant the two above aspects of the invention.

It is preferred that the number of primers used in one single reaction mixture is at least 7, but higher numbers are preferred such as at least 8, 10, 12, 14, 16, 20, 26, or at least 30.

It is expected that the number of primers in one single reaction mixture will be at most 50, but in certain situations the number will be at most 40 or even 35.

Preferred specific numbers of primers in one reaction mixture are apparent from the claims.

As used herein, the terms "chromosomal abnormality" and "chromosomal abnormalities" denote chromosomal sequences of nucleic acids which are usually not detectable in normal healthy subjects whereas these sequences are typically found in subjects suffering from diseases, having an increased risk of developing said diseases, or having well-defined chromosomal defects. Typically, such chromosomal abnormalities are translocations, inversions, deletions, duplications.

It follows that the chromosomal abnormality(ies) will normally be associated with a condition such as a disease (often malignant) or a chromosomal defect, or in other words, the chromosomal abnormality will be present in a significantly higher percent of subjects having the condition than in the average population.

In this connection, a "characteristic nucleic acid sequence" is a consecutive stretch of nucleotides which is comprised in the genome of a subject having a chromosomal abnormality and usually not in the "average" healthy subject. Further, the characteristic nucleic acid sequence is one the nucleotide sequence of which is uniquely tied to the chromosomal abnormality, *i.e.* it will not be found in any substantial number of nucleic acid samples from subjects which do not harbour the chromosomal abnormality.

By the term "molecular amplification procedure" is meant a *in vitro* procedure in which a nucleic acid sequence is multiplied by use of priming sequences ("primers") which anneal to a target sequence (the "template") and means for initiating and sustaining amplification of the extension products of the primers or complements thereof. Such methods are well-known in the art, but as exemplary can be mentioned the methods described in EP-0 200 362, EP-0 201 184, EP-A-0 368 906, EP-A-0 379 368, EP-A-0 540 693.

When referring to a "multiplex" molecular amplification procedure is, as well-known in the art, meant a molecular amplification procedure which comprises the use of at least three primers and which results in the amplification of at least two target sequences. In general, multiplex molecular amplification procedures are described in a number of patent publications, cf. e.g. EP-A-0364 255.

The preferred molecular amplification method according to the invention is multiplex PCR.

According to the methods of the invention, the primers used in the amplification procedure must be "distinct", by which term is meant nucleic acid primers which are not 100% identical in sequence and which furthermore will not, under the chosen amplification conditions, exhibit substantial mutual competition for annealing to a given target sequence.

Important embodiments of the methods of the invention are those wherein the sample of nucleic acids is derived from subjects in the form of cDNA. It will be understood that this requires the use of preceding method steps wherein cDNA is provided by employing reverse transcription of mRNA derived from the subjects and such a procedure thus limits the number of characteristic sequences to be detected to those which are actually transcribed in at least some of the subject's cells. On the other hand, the procedures for retrieving mRNA from cells are well-established in the art and involve relatively few problems in a standard setup.

It should be emphasized though, that the methods of the invention are in no way restricted to use of cDNA as template molecules in the amplification procedures. One interesting possibility will be to extract chromosomal DNA from the subject's cells and perform the multiplex molecular amplification either directly thereon or restriction fragments thereof. In this way it will be possible to detect chromosomal abnormalities which do not give rise to an apparent phenotype at the time of extraction but which nevertheless may be an important marker for the condition of interest.

It is, though, preferred that the molecular amplification procedure performed in the method of the invention utilises cDNA obtained by use of specific or non-specific cDNA primers in a separate molecular amplification procedure wherein the templates in the procedure are in the form of mRNA derived from the subject.

In standard schemes for obtaining mRNA, the cDNA primers used are normally non-specific, and the mRNA extraction is therefore "randomly" primed. The present inventor has discovered that markedly superior results are obtained in the detection phase when a mixture of specific cDNA primers are used for synthesis of cDNA from total RNA. In fact, the use of specific cDNA primers has given rise to an approximately 25 to 125

fold increase in sensitivity depending on the system in question.

It is therefore especially preferred that the cDNA primers are specific and in fact, the use of specific cDNA primers when coupled to subsequent multiplex molecular amplification procedures is in itself believed to be a novel approach which leads to a substantially higher sensitivity in the multiplex PCR reaction. Thus, another part of the invention is the combination of such specifically primed cDNA production with subsequent multiplex molecular amplification.

By the term "specific" when used in conjunction with cDNA primers is herein meant that the cDNA primers are predesigned to anneal to target RNA sequences which predominantly exist in RNA transcribed from the above-defined characteristic nucleic acid sequences.
According to the invention, the number of cDNA primers is preferably at least 20, such as at least 25, such as at least 30, such as at least 50, at least 100, at least 150, or at least 200.

When using cDNA (or any other source of template nucleic acids) for the multiplex molecular amplification procedure it is highly advantageous to avoid the need for exchange of media between the procedure for obtaining the template nucleic acids and the multiplex molecular amplification procedure. This can, according to the invention be done by ensuring that the conditions for obtaining cDNA (or other template nucleic acids) derived from the subject are compatible with the conditions of the molecular amplification procedure. In other words, the chemical composition of the medium for the molecular amplification procedure in the inventive method should be substantially the same as that of e.g. the cDNA synthesis procedure, whereas the melting point of any residual cDNA primers should be different from the melting point of the primers used in the multiplex molecular amplification procedure. In this way, it will be possible to

restrict the manipulation of test tubes to a minimum and thereby avoid contamination of the samples prior to the multiplex molecular amplification procedure.

A preferred embodiment of the invention is a method of multiplex molecular amplification, wherein said multiplex molecular amplification is a nested molecular amplification procedure such as a nested polymerase chain reaction. It is well-known that nested PCR enhances the specificity of any PCR reaction by excluding a large number of artefactual amplification products resulting from the initial round of PCR. Suitable nested PCR methods to be used according to the present invention are those described in USP 4,683,195, and especially the procedures described in EP-A-0 519 338, because these further ensures that no change of media or reactants between the individual steps in the nested molecular amplification procedure need be performed.

An important embodiment of the methods of the invention is a method wherein the chromosomal abnormality is the presence of a transcribed fusion gene. As explained above, a number of expressed fusion genes have been identified which are related in a highly significant manner to various malignant diseases of haematopoietic origin and therefore the detection of the presence of such fusion genes provides important and useful information of the prognosis of the subject, since certain of the malignancies are known to be susceptible to specific regimens of treatment. The presence of such a transcribed fusion gene is typically the result of an inversion, a deletion, a duplication, or activation of a proto-oncogene. Said activated proto-oncogene is typically selected from the group consisting of Hox-11 and evi-1 and others as presented in Rabbits 1994, which is incorporated by reference herein.

However, any genetic variant which is predominantly seen in e.g. malignant cells may be detected according to the invention, when the material subjected to the methods of the invention is not cDNA but for instance nucleic acid fragments

derived from a chromosomal source. In this way, a gene like c-myc, which is often overexpressed, may be detected and used as an indication of illness.

Accordingly, preferred embodiments of the methods of the invention are those wherein at least one of the chromosomal abnormalities is associated with a malignant neoplastic condition, especially a systemic neoplastic malignancy, since a relatively large number of these have been shown to be associated with e.g. expressed fusion genes.

According to the invention, such systemic neoplastic malignancies are selected from the group consisting of leukaemia such as acute leukaemia (AL), chronic leukaemia (CL), T-cell acute leukaemia (T-ALL), B-cell acute leukaemia (B-ALL), T-cell chronic leukaemia (T-CLL), B-cell chronic leukaemia (B-CLL), prolymphocytic leukaemia (PLL), acute undifferentiated leukaemia (AUL), acute myelogenous leukaemia (AML), chronic myelogenous leukaemia (CML), chronic myelomonocytic leukaemia (CMML), acute promyelocytic leukaemia (APL), pre-B-ALL, and pro-B-ALL;
lymphoma such as Burkitt's lymphoma (BL), non-Hodgkins lymphoma (NHL), Hodgkins lymphoma (HL), follicular lymphoma (FL), diffuse large cell lymphoma (DLCL), T-cell lymphoma, B-cell lymphoma; myelodysplasia; and myeloid.

The following chromosomal rearrangements have all been shown to be coupled to malignancies of the haematopoietic system:
dup(11q23) (dup exon 5-9/2);
dup(11q23) (dup exon 5-9/4);
inv(16)(p13;q22);
t(1;11)(p32;q23);
t(1;19)(q23;p13);
t(10;11)(p14;q23);
t(10;11)(p14;q23);
t(10;11)(p14;q23);
t(10;14)(q24;q11);
t(11;17)(q23;q21);

t(11;19)(q23;p13.1);
t(11;19)(q23;p13.3);
t(12;21)(p13;q22);
t(12;22)(p13;q11);
t(15;17)(q21;q22);
t(15;17)(q21;q22);
t(16;21)(p11;q22);
t(17;19)(q22;p13);
t(2;3)(p21;q26);
t(2;5)(p23;q35);
t(3;21)(q26;q22);
t(3;3)(q21;q26);
t(3;5)(q25.1;q34);
t(4;11)(q21;q23);
t(5;12)(q33;p13);
t(5;17)(q35;q22)
t(6;11)(q27;q23);
t(6;9)(p23;q34);
t(7;10)(q35;q24);
t(7;9)(q34;q32);
t(8;21)(q22;q22);
t(9;11)(q22;q23);
t(9;12)(q34;p13);
t(9;22)(q34;q11)
t(9;22)(q34;q11)
t(X;11)(q13;q23); and
$tal1^{d1-3}$ (40 kb deletion),

(The letter "t" used in such indications of chromosomal rearrangements denotes a translocation, "inv" denotes an inversion", and "dup" a duplication.)

In connection with these abnormalities, the following genes often become expressed: CBFβ/MYH11, SIL1/TAL1, MLL1, EVI-1, MLL1/AFX1, MLL1/AF1p, MLL1/AF1q, E2A/PBX1, E2A/HLF, EVI1, NPM/ALK, NPM/MLF, AML1/EVI1, MLL1/AF4, TEL/PDGfβ, NPM/RARα, DEK/CAN, SET/CAN, MLL1/AF6, HOX11, AML1/MTG8, MLL1/AF9, BCR/ABL, MLL1/AF10, MLL1/AF17, PLZF/RARα, MLL/ELL, MLL/ENL,

TEL/AML 1, PML/RARα, FUS/ERG, AML1/MDS, AML1/EAP, TEL/MN1, MLL exon 5-9/2, and MLL exon 5-9/4.

The above are thus the most preferred chromosomal abnormalities and genes which are detected in the methods of the invention, and primers (both cDNA and PCR) used in the methods of the invention should therefore be designed in order to specifically amplify sequences characteristic of these rearrangements.

Even though the main body of knowledge of the correlation between chromosomal rearrangements and malignancies is confined to the tumours of the haematopoietic system, knowledge do exist of similar correlations in other, solid tumours, cf. Rabbits, 1994. The methods of the invention are therefore also used to detect neoplastic conditions which are non-systemic neoplastic malignancies. Exemplary of such malignancies are non-systemic neoplastic malignancies selected from the group consisting of carcinoma, adenocarcinoma, liposarcoma, fibrosarcoma, chondrosarcoma, osteosarcoma, leiomyosarcoma, rhabdomyosarcoma, glioma, neuroblastoma, medullablastoma, malignant melanoma, neurofibrosarcoma, heamangiosarcoma, lymphangiosarcoma, malignant teratoma, dysgerminoma, seminoma, and choriocarcinoma. When the neoplastic disease is carcinoma it is preferably selected from the group consisting of carcinoma of the breast, bronchus, colorectum, stomach, prostate, ovary, lymphoid tissue, lymphoid marrow, uterus, pancreas, oesophagus, urinary bladder, kidney, or skin.

Especially interesting malignant neoplastic conditions are selected from the group consisting of papillary thyroid carcinoma, Ewing's sarcoma, liposarcoma, rhabdomyosarcoma, synovial sarcoma, and melanoma of soft parts, since all of these are positively associated with genomic rearrangements, cf. Rabbits 1994.

The sample of nucleic acids used in the methods of the invention is typically derived from cells of the bone marrow in the subject or from peripheral blood cells in the subject. This is especially interesting in the cases wherein the disease to detect is a malignant disease of the haematopoietic system, but also conditions characterized by chromosomal defects (e.g. Downs syndrome) may be detected this way. However, especially for the purpose of detecting the above-mentioned chromosomal defects, the sample may be derived from any other source in the subject, but interesting origins are placental cells, foetal cells, and amniotic fluid. A sample of 5 million mononuclear cells will normally be sufficient to deliver an amount of nucleic acid of 5 μg (between 3 and 8 μgr) RNA, however it depends on the growth rate of the cells. A 20 ml sample from the bone marrow generally corresponds to 5 μg (between 3 and 8 μg) RNA. Accordingly, as little as 10 ml of the sample may be sufficient for the method according to the present invention

In order to facilitate detection of amplified characteristic sequences, at least one of the primers used in the multiplex molecular amplification procedure may according to the invention be labelled. The label can be a radioactive label, a coloured label, a fluorescent label, a biotinyl group, an enzymatic group, a phosphate, an amin a tiol, or any other moiety which can be detected directly or indirectly. For instance, a biotinyl group may in itself be labelled, but it is also possible to detect the presence of the biotinylated nucleic acid fragment by reacting the mixture with labelled avidin or streptavidin.

In especially preferred embodiments of the methods of the invention, the primers are labelled with a fluorescent label or a coloured label. By using primers in the multiplex molecular amplification procedure which are both labelled and unlabelled it also becomes possible to use the degree of fluorescense in a quantitative way. If, for instance, a primer exists in two versions which are different labelled

e.g., a labelled and an unlabelled, and is used in differendt known amounts the amplification products will exhibit an average degree of labelling which correspons with the initial defined ratios of label due to the stochastic distribution of the labels in the amplified product. Hence, even though relatively few fluorescent labels of primers are known, it is possible to detect a large number of different amplified fragments when the above-indicated technique is used.

Instead of using labels it is of course possible to carefully choose primers which pairwise gives rise to amplified products of different lengths. The presence or absence of amplified products is then detected by use of various methods which are able to detect the amplified fragments on basis of their size/sequence, methods such as gel electrophoresis, sequence analysis, HPLC, FPLC, flouresence spectofotometri and other suitable chromatograhphic methods.

Alternatively, a labelled means for detecting the amplified products may be used, such as other nucleic acid fragments which will hybridize to the product and thereafter be detectable by virtue of the label. Such methods are well-known in the art.

Even though it according to the invention is possible to use a large number of primers in the same reaction vessel, there is an upper limit beyond which the amplification procedure becomes too unstable and unreliable. In an important embodiment of the invention, wherein the sample of nucleic acids is subjected to at least two multiplex molecular amplification as defined herein, *i.e.* the sample(s) derived from the subject is split into several aliquots which each are subjected to a multiplex molecular amplification procedure according to the invention. It is preferred (in view of the reduced time consumption) that the at least two multiplex molecular amplification procedures are carried out in parallel, and it is especially preferred that the at least two multiplex molecular amplification procedures are carried out under substan-

tially the same conditions with respect to physical parameters and timing; the latter preferred embodiment has the advantage of allowing the use of e.g. the same reaction buffers (with the exception of primers) and the same thermocycling scheme for all aliquots. In essence, all reactions are thus performed in the same thermocycler.

The exact number of different amplification procedures the sample is subjected to may vary, but is preferably at least 3, such as at least 4, 5, 6, 7, 8, 9, 10, 11, 12, or at least 15. Higher numbers may be necessary, depending on the number of families of chromosomal abnormalities need be detected.

The internal standard used in one of the methods of the invention is preferably a cDNA molecule derived from the subject and most preferably said cDNA molecule is obtained by use of specific or non-specific cDNA primers in a molecular amplification procedure wherein the templates in the procedure are in the form of mRNA derived from the subject.

In fact, by deriving the internal standard from the very cells which are the source of the sample of nucleic acids derived from the subject and by using the same type of steps to obtain the internal standard as the ones used for obtaining the nucleic acids derived from the subject, a reliable indication is obtained of the correct execution of all steps leading up to and including the multiplex molecular amplification procedure. The demonstration of the presence of the internal standard in the final mixture of amplified products will in such a setup indicate that all previous steps have been performed satisfactorily, whereas the demonstration of the absence of the standard will indicate that the assay should be repeated.

Hence, in the case where the sample of nucleic acids derived from the subject is constituted of cDNAs, the internal standard is also cDNA which has been obtained in parallel to the

other cDNAs, including the molecular amplification procedure leading to the provision of the other cDNAs.

However, less reliable but nevertheless satisfactory confirmation of the correct execution of the various process steps may for instance be obtained by one of the following schemes:

1) A RNA fragment of known sequence is added to the total RNA mixture from which mRNA is extracted, whereafter the subsequent products of the known RNA are obtained from reverse transcription PCR; in this way, all steps but the total RNA extraction are confirmed;

2) A nucleic acid fragment of known sequence is added to the multiplex molecular amplification mixture together with appropriate primers; in this way the multiplex molecular amplification procedure in itself can be confirmed.

It is however preferred to use the internal standard "all the way" and in such embodiments of the invention, a cDNA molecule constituting the internal standard corresponds to a constitutively expressed RNA fragment. In the present examples is used a sequence from the constitutively expressed gene E2A, but any gene which is constitutively expressed in the cells of interest may be used as target. In embodiments where the nucleic acids constituting the sample are derived directly from chromosomal DNA, any "normal" gene sequence may be employed as internal standard.

A crucial factor in the inventive methods are the choice of primers used in the multiplex molecular amplification procedure. In general, the primers are designed using standard software known to the skilled person, and a number of criteria must be met by the primers in the reaction mixture:

1) primers must hybridize to their respective target sequences at or below substantially the same temperature, preferable within a temperature diffenrence of 5°C (they

should have the same melting point); in the setup reported in the following examples, the melting point has been chosen to be approximately 70°C,

2) primers must be substantially specific for their respective target sequences, meaning that they will not initiate polymerization from other template sequences than "their own" and that they are not capable of hybridizing with each other. This specificity is obtained by the fact that the primers are completely complementary to the target sequence, however up to 3 point differencies (mutations) may still result in a specific priming.

3) primers should exhibit substantially no intramolecular hybridization, or in other words, there must be a minimum of secondary structure in each primer, that is normally the case when the delta G is above -1 within the primer,

4) primers must have a higher melting point in the 5'-end than in the 3'-end, *i.e.* they have a high internal stability in the 5' end and a relatively low stability in the 3' end, the difference in melting point is preferable above 1°C, such as above 2°C, preferable above 3°C, such as above 4°C and more preferred above 5°C, such as above 6°C, howver, the exact difference may also depends on other desired properties of the primers.

5) no two primers are, in the molecular amplification procedure, capable of together initiating and sustaining amplification of nucleic acid fragments in the sample which correspond to normally occurring sequences not associated with a condition in the subject,

6) no primer should preferably contain more than 5 consecutive guanidyl residues, such as not more than 3 guanidyl residues.

7) they should exhibit substantially no intermolecular hybridization, which may be obtained for the primer dimer having a delta G being above -10.

A further restraint on the choice of primers is that they should pairwise give rise to fragments of different lengths when the molecular amplification procedure is the one resulting in the amplified products to be detected (*i.e.* the last molecular amplification procedure, e.g. the second PCR in a nested PCR) and the procedure is one wherein the amplified products are distinguished by their length/sequence. In such a situation the amplified fragment should typically have a length of between 100-400 bp.

It will be understood that the precise choice of primers can be varied in an almost indefinite number of ways as long as they conform with the sequences of e.g. the fusion genes to be detected and the above criteria are met. However, as PCR primers the primers having any of SEQ ID NOs 33-177 are currently being especially preferred. As cDNA primers, the primers having any of SEQ ID NOs 1-32 and 178-182 are currently being preferred.

In a further aspect, the invention also relates to a kit comprising 7 mutually distinct primers. The kit may comprise any disired combination of primers for the methods for detection of the presence or absence of chromosomal abnormalities according to the present invention. Accordingly, the kit may comprise primers selected from the group of cDNA primers consisting of SEQ ID NO: 1 through SEQ ID NO: 32 and SEQ ID NO: 178 through SEQ ID NO: 182 and of PCR primers selected from SEQ ID NO: 33 trough SEQ ID NO: 177. The kit according to the present invention may also comprise additives such as buffers, enzymes, and stabilizing agents known in the art.

In a preferred embodiment, the primers are attached to a surface of a device such as a well, e.g of a multiplate, a cappilary tube, a stick, or a bead (such as a magnetic bead).

In this connection, the primers may be dried or in other any suitable form including being contained in a polymer vehicle. In a further embodiment, the primers in the kit are in a liquid form contained in e.g. a tube or well.

The above-mentioned specific primers also in themselves constitute another aspect of the invention as do homologues thereof which will perform equally effectively in the PCR amplifications described herein or as cDNA primers.

LEGENDS TO FIGURES

Figure 1. Setup of the multiplex PCR amplification reaction. The figure shows representative results of gel electrophoreses of nucleic acid samples from one patient subjected to 8 multiplex nested PCR amplifications, each multiplex nested PCR using the primer mixes 1-8. The upper lane (lane 1) represents molecular weight markers. The band which is present in lanes 1-8 is the internal standard, while the second band in lane 5 (primer mix 4) is a detected chromosomal abnormality as highlighted in bold.

Figs. 2A and 2B. The figure shows gel electrophoreses of nucleic acid samples from 18 individual patients, subjected to multiplex nested PCR amplification using the primer mixes 1-8. In each patient one or more chromosomal abnormalities has been detected by the PCR giving rise to a typical and readily identifiable pattern of bands. Above each panel, representing one patient, the actual chromosomal abnormality has been specified.

Figure 3. The figure shows samples positive in the multiplex analysis reanalyses with individual primer sets. The upper three panels each represent gel electrophoreses of multiplex nested PCR performed on nucleic acid samples from one patient. From each of these gels one lane shows that the individual harbours a chromosomal abnormality, the precise nature and variant of which cannot be readily determined. In

order to clearly identify this abnormality nucleic acids from each patient were subjected to another round of PCR (lower three panels) this time using individual primer sets able to determine the nature of the fusion genes involved.

EXAMPLE 1

Detection of chromosomal abnormalities in patient samples using multiplex PCR

MATERIALS AND METHODS

Patient Samples and Cell Lines

Bone marrow or peripheral blood samples were fractionated on a Ficoll gradient and cryopreserved before use. The cell lines Karpas-299, ML-2, Mono-Mac-6, NB-4, 697, JOSK-I, NALM-6 and RPMI-8402 were obtained from DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) - (DSM accession numbers 31, 15, 124, 207, 42, 155, 128 and 290 respectively). The cell lines RS4;11 and MV-4-11 were obtained from the American Type Culture Collection (Rockville, MD) (ATCC accession numbers CRL 1873 and CRL 9591, respectively). The cell line HAL-01 is described in Ohyashiki *et al.* (1991), Leukaemia **5**: pp. 322-331. The cell lines were all cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. The medium for the cell line Mono-Mac-6 was supplemented with 9 μg/mL bovine insulin.

RNA Preparation

Total RNA was prepared either by the guanidinium thiocyanate-phenol chloroform method [Chomczynski, Anal. Biochem 162:156, 1987] or by using a RNeasy Kit (Quiagen) according to the manufacturer's instructions. The RNA solution was subsequently treated with 0.1 unit/μL RNase-free DNase (Boehringer) in 50 mmol/L Tris-HCl, pH 8.0, 10 mmol/L $MgCl_2$ at 37°C for 30 minutes. After the DNase treatment EDTA, pH 8.0

was added to 10 mmol/L and the RNA solution was extracted once in phenol/chloroform 1:1, sodium-acetate added to 200 mmol/L and precipitated with 1 volume of isopropanol. The RNA was pelleted in an eppendorf centrifuge at 13.000 rpm for 30 minutes and washed with 80% ethanol. The RNA was resuspended in 25 µL DEP ddH_2O and 5 µL withdrawn for quantification on a Genequant (Pharmacia). Subsequently the RNA was diluted to 0.1 µg/µL and stored until use at -80°C in 10 µL aliquots.

Reverse Transcriptase PCR

One microgram of total RNA was incubated for 5 minutes with a mixture of translocation-specific-cDNA-primers (2.5 pmol of each) and then reverse transcribed to cDNA by incubation at 37°C for 45 minutes in a total volume 25 mL containing 20 units RNase inhibitor (Boehringer), 1 mmol/L of each dNTP, 10 mmol/L dithiothreitol, 1XRT buffer (50 mmol/L Tris-HCl pH 8.3, 75 mmol/L KCl, 3 mmol/L $MgCl_2$), and 400 units Moloney murine leukaemia virus reverse transcriptase (BRL, Bethesda, MD). At the end of the incubation, the cDNA reaction mixture was diluted with ddH_2O to 50 µL. The PCR amplification was performed as 8 parallel nested (two round) multiplex reactions in a Perkin Elmer 9600 thermocycler. Five µL of diluted cDNA reaction was added to each of eight 20 µL multiplex mixtures which contained 1.1 X PCR buffer (10 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$), 0.2 mmol/L of each dNTP, 12,5 pmol of each primer and 1.5 unit AmpliTaq-Gold polymerase (Perkin Elmer). The first PCR reaction time consisted of an initial activation of the polymerase at 95°C for 15 minutes, followed by 25 cycles of PCR amplification (annealing at 58°C for 30 seconds, elongation at 72°C for 1 minute, and denaturation at 95°C for 30 seconds). After the first round of PCR, a 1 µL aliquot from each of the 8 PCR reactions was transferred to eight 24 µL second round multiplex mixtures which contained 1 X PCR buffer (10 mmol/L Tris-HCl pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$), 0.2 mmol/L of each dNTP, 5-12.5 pmol of each primer and 1.5 unit AmpliTaq-Gold polymerase. The second PCR reaction time consisted of an

initial activation of the polymerase at 95°C for 15 minutes, followed by 20 cycles of PCR amplification (annealing at 58°C for 30 seconds, elongation at 72°C for 1 minute, and denaturation at 95°C for 30 seconds) followed by a 10 minutes extension at 72°C. Fifteen μL of the PCR reactions were electrophoresed in a 1.5% agarose gel for 60 minutes at 100V and stained with ethidiumbromide. Positive samples were reanalyzed to verify/determine the translocation(s) by performing cDNA and nested PCR with the individual primer sets using the same conditions as for the multiplex PCR except that only 0.75 unit/reaction of AmpliTaq-Gold polymerase was used. Positive samples with limiting amount of RNA were reanalyzed by performing only the second round of PCR with the individual primer sets using 1 μL from the first round multiplex PCR as template. This analysis was performed with and without the internal control primers and translocations were confirmed by DNA sequence analysis. Negative controls without DNA template were included for all PCR reactions mixtures. To minimize the risk of contamination filtertips were used in all steps and four different laboratories with indigenous pipettes were used for the preparation of stock solutions, the RNA preparation and cDNA synthesis/setup of first PCR, the first to second PCR transfer, and the gel electrophoresis.

Primer design and DNA sequencing

All PCR oligonucleotide primers were designed using the Windows primer analysis software OLIGO version 5.0 (National Biosciences Inc., Plymouth, MN) using data from the EMBL DNA database. Oligonucleotide primers were supplied HPLC purified from DNA Technology, Science Park, DK-8000 Aarhus. DNA sequencing was performed on agarose gel purified PCR fragments using a Taq DyeDeoxy Terminator Sequencing kit (Perkin Elmer) on an automated 373A DNA sequencer (Applied Biosystems, Foster City, CA). Both strands of the PCR fragments were sequenced. The cDNA primers were:

1. 12-mer: CBFBMYHA:1752L12,
5' AgC TgC TTg ATg 3' (SEQ ID NO: 1).
2. 12-mer: CBFBMYHA:1033L12,
5' CTg CTg ggT gAg 3' (SEQ ID NO: 2).
3. 12-mer: ALL1AF1:4220L12,
5' ATg ggA gCT CAg 3' (SEQ ID NO: 3).
4. 12-mer: ALL1AF1Q:4355L12,
5' Agg gCT TTT gAg 3' (SEQ ID NO: 4).
5. 11-mer: E2APRL:764L11,
5' CCC TCC AgA Ag 3' (SEQ ID NO: 5).
6. 12-mer: ALKNPM:714L12,
5' CAg CgA ACA ATg 3' (SEQ ID NO: 6).
7. 12-mer: AML1EVI:2459L12,
5' CCC ATC CAT AAC 3' (SEQ ID NO: 7).
8. 12-mer: ALL1AF4:4349L12,
5' TTC CTT gCT gAg 3' (SEQ ID NO: 8).
9. 12-mer: TELPDGF:1003L12,
5' CTg CAg gAA ggT 3' (SEQ ID NO: 9).
10. 12-mer: DEKCAN:1446L12,
5' TTg gCT ggT ACT 3' (SEQ ID NO: 10).
11. 12-mer: ALL1AF6:4150L12,
5' CCg ATC ATC TTT 3' (SEQ ID NO: 11).
12. 12-mer: AML1MTG8:2460L12,
5' gTg CgA ACT CTT 3' (SEQ ID NO: 12).
13. 12-mer: AF9:1536L12,
5' CTg CCA TCA CTT 3' (SEQ ID NO: 13).
14. 12-mer: ALL1AF9:4184L12,
5' gCA TCC AgT TgT 3' (SEQ ID NO: 14).
15. 12-mer: ABL:797L12,
5' gCT gCC ATT gAT 3' (SEQ ID NO: 15).
16. 12-mer: ALLAF10A:3997L12,
5' CCA Ctg CCT CTC 3' (SEQ ID NO: 16).
17. 12-mer: AF10:1150L12,
5' ACC TgA gCT gTg 3' (SEQ ID NO: 17).
18. 13-mer: AF10:750L13,
5' gTA gCC ACA gTA T 3' (SEQ ID NO: 18).
19. 11-mer: AF17:1965L11,
5' gAC ACC ggA Ag 3' (SEQ ID NO: 19).

20. 12-mer: BCR1:2094L12,
5' Cgg TCg TTT CTC 3' (SEQ ID NO: 20).

21. 12-mer: ALL1ENL:4271L12,
5' TCT CCA CgA AgT 3' (SEQ ID NO: 21).

22. 12-mer: ALLELL:4341L12,
5' CCA gCC TTg ATg 3' (SEQ ID NO: 22).

23. 12-mer: EWSERG:1071L12,
5' TgT Agg CgT AgC 3' (SEQ ID NO: 23).

24. 12-mer: E2AHLF:1726L12,
5' ggC CTC ATA CTT 3' (SEQ ID NO: 24).

25. 11-mer: E2A:1960L11,
5' GCT TCG CTC AG 3' (SEQ ID NO: 25).

26. 12-mer: AML1EVI:4905L12,
5' TAA ggC TgC TCT 3' (SEQ ID NO: 26).

27. 12-mer: TELAML1:1365L12
5' Cgg Tag CAT TTC 3' (SEQ ID NO: 27).

28. 11-mer: TAL1:263L11,
5' Ccg TcC CTC TA 3' (SEQ ID NO: 28).

29. 12-mer: AFX1:70L12,
5' Aag TgC CAA Cag 3' (SEQ ID NO: 29).

30. 11-mer: HOX11:897L11,
5' TgC TgC CTC TC 3' (SEQ ID NO: 30).

31. 13-mer: ALL1:417L13,
5' TTT ggT CTC TgA T 3' (SEQ ID NO: 31).

32. 12-mer:MLF1:320L12
5' Tgg TCT ggA Aag 3' (SEQ ID NO: 32).

178. ENL:1405L12
5' GCCTGACACCTT 3' (SEQ ID NO: 178).

179. ALL1:3275L11
5' CTGCCCACACC 3' (SEQ ID NO: 179).

180. MN1:5065L12
5' GCCACTAAGCAG 3' (SEQ ID NO: 180).

181. EAP:1012L13
5' TAATCCTCGTCTT3' (SEQ ID NO: 181).

182. AML1EVI:2820L13
5' GTCCTCTTCAACC 3' (SEQ ID NO: 182).

The following tables recite the composition of the 16 different reaction mixtures used in the 8 nested PCR reactions, the interrelationship between the PCR primers and the chromosomal rearrangement to be detected, and the resulting PCR fragments ("NP" mixes are stock solutions adapted for detection of one single rearrangement, whereas "R mixes" are the combinations of NP mixes used in the multiplex nested PCR reactions, finally, the M-mixes are an alternative to the R-mixes, the difference is that the M-mixes detects 5 additional fusion genes, but not the activiation of HOX11 and EVY1 as tested for in the R-mixes). The control primer set (NP-mix No. 41) is included in the tests as shown.

R-mix no.	PCR Primers, 1st PCR	SEQ ID NO:	PCR mix pmol/µl	PCR Primers 2nd PCR	SEQ ID NO:	PCR mix pmol/µl
R1	CBFBMYHC:267U22	81	5	CBFBMYH:344U21	129	12.5
	CBFBMYHC:752L22	39	5	CBFBMYHC:595L19	125	12.5
	CBFBMYHA:919L24	58	5	CBFBMYHA:868L20	124	12.5
	ALLAF10A:3730U20	49	5	ALL1AF4:3751U20	114	12.5
	ALL1:3955U24	36	5	ALL1:3996U24	88	12.5
	AFX1:29L20	68	5	AFX1:5L24	118	12.5
	ALL1AF6:4074L21	50	5	ALL1AF6:4037L22	109	12.5
	ALL1ELL:4236L23	34	5	ALL1ELL:4191L22	132	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
R2	ALLAF10A:3730U20	49	5	ALL1AF4:3751U20	114	12.5
	ALL1:3955U24	36	5	ALL1:3996U24	88	12.5
	ALL1AF1:4048L22	65	5	ALL1AF1:4031L21	130	12.5
	AF17:1937L21	51	5	ALL1AF17:4009L23	119	12.5
	ALLAF10A:3968L23	56	5	ALLAF10A:3932L21	134	12.5
	ALLAF10B:4031L22	79	5	ALLAF10B:3996L23	131	12.5
	AF10:728L22	76	5	AF10:685L21	115	12.5
	ALL1:391L23	80	5	ALL1:313L28	110	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
R3	E2APRL:220U21	78	5	E2APRL:673L21	95	12.5
	E2APRL:696L18	61	5	E2A:1173U19	103	12.5
	SIL:24U19	52	5	SIL:83U20	98	12.5
	TAL1:203L21	55	5	TAL1:179L20	86	12.5
	E2AHLF:1685L20	62	5	E2AHLF:1543L20	100	12.5
	TELAML1:871U23	44	5	TELAML1:944U23	104	12.5
	TELAML1:1335L23	37	5	TELAML1:1216L21	87	12.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
R4	AML1EVI:1897U21	60	5	AML1MGT8:1895U20	128	12.5
	AML1EVI:2375L24	53	5	AML1EVI:2345L21	97	12.5
	HOX11:590U20	67	5	HOX11:617U22	106	12.5
	HOX11:857L21	70	5	HOX11:810L19	113	12.5
	AML1MGT8:2437L23	73	5	AML1MGT8:2226L22	102	12.5
	TLSERG:649U19	69	5	TLS:690U19	93	12.5
	EWSERG:979L22	72	5	TLSERG:945L19	116	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		

R-mix no.	PCR Primers, 1st PCR	SEQ ID NO:	PCR mix pmol/µl	PCR Primers 2nd PCR	SEQ ID NO:	PCR mix pmol/µl
R5	ALLAF10A:3730U20	49	5	ALL1AF4:3751U20	114	12.5
	ALL1:3955U24	36	5	ALL1:3996U24	88	12.5
	ALL1AF4:4321L29	57	5	ALL1AF4:4291L25	122	12.5
	ALL1AF9:4143L24	48	5	ALL1AF9:4092L24	108	12.5
	AF9:1498L22	77	5	AF9:1466L26	105	12.5
	ALL1AF1Q:4211L22	64	5	AF1Q:580L20	127	12.5
	ALL1ENL:4215L22	42	5	ALL1ENL:4164L19	112	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
R6	BCR1ABL:1698U19	54	5	BCR1ABL:1777U19	85	12.5
	BCR2ABL:3060U23	59	5	BCR2ABL:3128U22	91	12.5
	BCR1ABL:2093L20	83	5	BCR1ABL:2074L23	84	12.5
	TELPDGF:309U21	63	5	TELPDGF:343U24	99	12.5
	TELPDGF:834L22	45	5	TELPDGF:642L22	94	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
R7	DEKCAN:870U24	74	5	DEKCAN:892U21	89	12.5
	DEKCAN:1422L21	66	5	SETCAN:925L20	120	12.5
	SETCAN:468U23	43	5	SETCAN:552U24	126	12.5
	AML1EVI:4331U23	47	5	AML1EVI:4509U21	101	12.5
	AML1EVI:4866L21	75	5	AML1EVI:4746L25	123	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
R8	PLZFRARA:1092U21	46	5	PLZFRARA:1252U21	121	12.5
	BCR1:1338U19	40	5	BCR1:1497U21	111	12.5
	BCR3:988U19	71	5	BCR3:1057U20	92	12.5
	BCR3:1460L19	33	5	BCR3:1428L22	96	12.5
	ALKNPM:200U25	35	5	ALKNPM:313U21	117	12.5
	ALKNPM:627L21	41	5	ALKNPM:590L19	107	12.5
	MLF1:235L27	38	5	MLF1:192L28	133	12.5
	E2APRL:220U21	78	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		

NP-mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID NO:	PCR-Primers N2 (2nd PCR)	SEQ ID NO:	PCR Fragment(s)	Pos. Controle
2	inv(16)(p13;q22)	CBFβ (16q22) MYH11(16p13)	CBFBMYHC:267U22 CBFBMYHC:752L22	81 39	CBFBMYH:344U21 CBFBMYHC:595L19	129 125	174 bp to 663 bp	Pt.
4	inv(16)(p13;q22)	CBFβ (16q22) MYH11(16p13)	CBFBMYHC:267U22 CBFBMYHA:919L24	81 58	CBFBMYH:344U21 CBFBMYHA:868L20	129 124	544 bp 337 bp	No
45	tal1^{d1-3} (40 kb deletion)	SIL1(1p34) TAL1(1p34)	SIL:24U19 TAL1:203L21	52 55	SIL:83U20 TAL1:179L20	98 86	183 bp	RPMI
49	dup(11q23) dup exon 2-5/8	ALL1(11q23)	ALLAF10A:3730U20 ALL1:391L23 ALL1:3955U24	49 80 36	ALL1AF4:3751U20 ALL1:313L28 ALL1:39996U24	114 110 88	244 bp	Pt
46	t(X;11)(q13;q23)	AFX1(X;q13) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AFX1:29L20	49 36 65	ALL1AF4:3751U20 ALL1:3996U24 AFX1:5L24	114 88 118	235 bp to 449 bp	Cell line: Karpas 45
5	t(1;11)(p32;q23)	AF1p(1p32) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF1:4048L22	49 36 65	ALL1AF4:3751U20 ALL1:3996U24 ALL1AF1:4031L21	114 88 130	187 bp to 301 bp	No
6	t(1;11)(q21;q23)	AF1q(1q21) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF1Q:4211L22	49 36 64	ALL1AF4:3751U20 ALL1:3996U24 AF1Q:580L20	114 88 127	287 bp to 368 bp	No
8	t(1;19)(q23;p13)	PBX1(1q23) E2A(19p13)	E2APRL:220U21 E2APRL:696L18	78 61	E2A:1173U19 E2APRL:673L18	95 103	376 bp	Pt+Cell line: 697
9	t(2;5)(p23;q35)	ALK(2p23) NPM(5q35)	NPMALK:200U25 NPMALK:627L21	35 41	NPMALK:313U21 NPMALK:590L19	117 107	302 bp	Pt+ Karpas 299
42	t(3;3)(q21;q26) t(2;3)(p21;q26) t(3;21)(q26;q22)	Activation of EVI1(3q26)	AML1EVI:4331U23 AML1EVI:4866L21	47 75	AML1EVI:4509U21 AML1EVI:4746L25	101 123	262 bp	Pt+Cell line:

NP-mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID NO:	PCR-Primers N2 (2nd PCR)	SEQ ID NO:	PCR Fragment(s)	Pos. Controle
50	t(3;5)(q25.1;q34)	MLF(3q25.1) NPM(5q34)	NPMALK:200U25 MLF1:235L27	35 38	NPMALK:313U21 MLF1:192:L28	117 133	289+333 362+406	
11	t(3;21)(q26;q22)	EVI-1(3q26) AML1(21q22)	AML1EVI:1897U21 AML1EVI:2375L24	60 53	AML1MGT8:1895U20 AML1EVI:2345L21	128 97	446 bp	Cell line: SKHI
12	t(4;11)(q21;q23)	AF4(4q21) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF4:4321L29	49 36 57	ALL1AF4:3751U20 ALL1:3996U24 ALL1AF4:4291L25	114 88 122	75 bp to 321 bp	Cell line: RS4;11 MV4;11
13	t(5;12)(q33;p13)	PDGFβ(5q33) TEL(12p13)	TELPDGF:309U21 TELPDGF:834L22	63 45	TELPDGF:343U24 TELPDGF:642L22	99 94	321 bp	
51	t(5;17)(q35;q22) S-, L-forms	NPM(5q35) RARA(17q21)	NPMALK:200U25 BCR3:1460L19	35 33	NPMALK:313U21 BCR3:1428L22	117 96	105 bp 234 bp	
14	t(6;9)(p23;q34)	CAN(9q34) DEK(6p23)	DEKCAN:870U24 DEKCAN:1422L21	74 66	DEKCAN:892U21 SETCAN:925L20	89 120	320 bp	Pt
15	?t(9;9)	CAN(9q34) SET(9q34)	SETCAN:468U23 DEKCAN:1422L21	43 66	SETCAN:552U24 SETCAN:925L20	126 120	393 bp	No
16	t(6;11)(q27;q23)	AF6(6q27) ALL(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF6:4074L21	49 36 50	ALL1AF4:3751U20 ALL1:3996U24 ALL1AF6:4037l22	114 88 109	199 bp to 313 bp	Pt+Cell line: ML-2
48	t(7;10)(q35;q24) t(10;14)(q24;q11)	Activation of HOX11(10q24)	HOX11:590U20 HOX11:857L21	67 70	HOX11:617U22 HOX11:810L19	106 113	212 bp	Pt+RPMI
17	t(8;21)(q22;q22)	MTG8(8q22) AML1(21q22)	AML1EVI:1897U21 AML1MGT8:2437L23	60 73	AML1MGT8:1895U20 AML1MGT8:2226L22	128 102	353 bp	Pt+Cell line: Kasumi-1
18A	t(9;11)(q22;q23)	AF9(9q22) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AF9:1498L22	49 36 77	ALL1AF4:3751U20 ALL1:3996U24 AF9:1466L26	114 88 105	208 bp to 322 bp	Cell line: Mono Mac 6

NP-mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID NO:	PCR-Primers N2 (2nd PCR)	SEQ ID NO:	PCR Fragment(s)	Pos. Controle
18B	t(9;11)(q22;q23)	AF9(9q22) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF9:4143L24	49 36 48	ALL1AF4:3751U20 ALL1:3996U24 ALL1AF9:4092L25	114 88 108	254 bp to 368 bp	Cell line: Mono Mac 6
43	t(9;12)(q34;p13)	ABL(9q34) TEL(12p13)	TELPDGF:309U21 BCR1ABL:2093L20	63 83	TELPDGF:343U24 BCR1ABL:2074L23	99 84	366 bp	No
19	t(9;22)(q34;q11) type e1a2	ABL(9q34) BCR(22q11)	BCR1ABL:1698U19 BCR1ABL:2093L20	54 83	BCR1ABL:1777U19 BCR1ABL:2074L23	85 84	320 bp	Pt
20	t(9;22)(q34;q11) type b2a2+b3a2	ABL(9q34) BCR(22q11)	BCR2ABL:3060U23 BCR1ABL:2093L20	59 83	BCR2ABL:3128U22 BCR1ABL:2074L23	91 84	472 bp 397 bp	Pt Pt
21A	t(10;11)(p14;q23) A+C type	AF10(10p14) ALL1(11q23)	ALLAF10:3730U20 ALL1:3955U24 ALLAF10A:3968L23	49 36 56	ALLAF4:3751U20 ALL1:3996U24 ALLAF10A:3932L21	114 88 134	202 bp to 389bp	Pt
21B	t(10;11)(p14;q23) B+D type	AF10(10p14) ALL1(11q23)	ALLAF10:3730U20 ALL1:3955U24 ALLAF10B:4031L22	49 36 79	ALLAF4:3751U20 ALL1:3996U24 ALLAF10B:3996L23	114 88 131	270 bp to 367 bp	Pt
21E	t(10;11)(p14;q23) E type	AF10(10p14) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AF10:728L22	49 36 76	ALL1AF4:3751U20 ALL1:3996U24 AF10:685L21	114 88 115	154 bp to 268 bp	No
22	t(11;17)(q23;q21) A	ALL1(11q23) AF-17(17q21)	ALLAF10A:3730U20 ALL1:3955U24 AF17:1937L22	49 36 51	ALL1AF4:3751U20 ALL1:3996U24 ALL1AF17:4009L23	114 88 119	284 bp	No
23	t(11;17)(q23;q21) B	PLZF(11q23) RARA(17q21)	PLZFRARA:1092U21 BCR3:1460L19	46 33	PLZFRARA:1252U21 BCR3:1428L22	121 96	315 bp 402 bp	Pt
24	t(11;19)(q23;p13.3)	ALL1(11q23) ENL(19p13)	ALLAF10A:3730U20 ALL1:3955U24 ALL1ENL:4215L22	49 36 42	ALL1AF4:3751U20 ALL1:3996U24 ALL1ENL:4164L19	114 88 112	73 bp to 187 bp	Pt: (HB1119+ KOCL33 ect.

NP-mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID NO:	PCR-Primers N2 (2nd PCR)	SEQ ID NO:	PCR Fragment(s)	Pos. Controle
25	t(11;19)(q23;p13.1)	ALL1(11q23) ELL(19p13)	ALLAF10A:3730U20 ALL1:3955U24 ALL1ELL:4236L23	49 36 34	ALL1AF4:3751U20 ALL1:3996U24 ALL1ELL:4191L22	114 88 132	217 bp to 421 bp	Pt
44	t(12;21)(p13;q22)	TEL(12p13) AML1(21q22)	TELAML1:871U23 TELAML1:1335L23	44 37	TELAML1:944U23 TELAML1:1216L21	104 87	293 bp 332 bp	Pt
30	t(15;17)(q21;q22) type V+L	PML(15q22) RARA(17q21)	BCR1:1338U19 BCR3:1460L19	40 33	BCR1:1497U21 BCR3:1428L22	111 96	427 bp	Pt+Cell line: NB4
31	t(15;17)(q21;q22) type S	PML(15q22) RARA(17q21)	BCR3:988U19 BCR3:1460L19	71 33	BCR3:1057U20 BCR3:1428L22	92 96	393 bp	Pt
32	t(16;21)(p11;q22)	FUS(16p11) ERG(21q22)	TLSERG:649U19 EWSERG:979L22	69 72	TLS:690U19 TLSERG:945L19	93 116	274 bp	Pt+ (UTP-L12)
33	t(17;19)(q22;p13)	HLF(17q22) E2A(19p13)	E2APRL:220U21 E2AHLF:1685L20	78 62	E2A:1173U19 E2AHLF:1543L20	103 100	390 bp	Cell line: HAL-01
41	Positive controle	E2A(19p13)	E2APRL:220U21 E2A:1883L22	78 82	E2A:1173U19 E2A:1844L19	103 90	694 bp	All Pts. and cell lines

R-mix no.	Chromosomal Rearrangement	genes	PCR primers (1st PCR)	SEQ ID NO:	PCR primers (2nd PCR)	SEQ ID NO:	PCR fragments	Comments
R1	inv(16)(p13;q22)	CBFβ(16q22) MYH11(16p13)	CBFBMYHC:267U22 CBFBMYHC:762L22 CBFBMYHA:919L24	81 39 58	CBFBMYH:344U21 CBFBMYHC:595L19 CBFBMYHA:868L20	129 125 124	174 bp to 663 bp	7 variants
(NP: 2, 4, 16, 29, 46)	t(X;11)(q13;q23)	AFX1(Xq13) ALL(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AFX1:29L20	49 36 68	ALL1AF4:3751U20 ALL1:3996U24 AFX1:5L24	114 88 118	235 bp to 449 bp	3 variants
	t(6;11)(q27;q23)	AF6(6q27) ALL(11q23)	ALL1AF6:4074L21	50	ALL1AF6:4037l22	109	199 bp to 313 bp	3 variants
	t(11;19)(q23;p13.1)	ALL1(11q23) ELL(19p13)	ALL1ELL:4236L23	34	ALL1ELL:4191L22	132	157 bp to 421 bp	6 variants
R2	t(1;11)(p32;q23)	AF1p(1p32) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF1:4048L22	49 36 65	ALL1AF4:3751U20 ALL1:3996U24 ALL1AF1:4031L21	114 88 130	187 bp to 301 bp	3 variants
(NP: 5, 22, 21A, 21B, 21E, 49)	t(11;17)(p23;q21) A	ALL1(11q23) AF17(17q21)	AF17:1937L22	51	ALL1AF17:4009L23	119	284 bp	
	t(10;11)(p14;q23) type A+B+C+D+E	AF10(10p14) ALL1(11q23)	ALLAF10A:3968L23 ALLAF10B:4031L22 AF10:728L22	56 79 76	ALLAF10A:3932L21 ALLAF10B:3996L23 AF10:685L21	134 131 115	164 bp to 389 bp	13 variants
	Dup(11q23)	ALL1(11q23)	ALL1:391L23	80	ALL1:313L28	110	244 bp	

R-mix no.	Chromosomal Rearrangement	genes	PCR primers (1st PCR)	SEQ ID NO:	PCR primers (2nd PCR)	SEQ ID NO:	PCR fragments	Comments
R3	t(1;19)(q23;p13)	PBX1(1q23)	E2APRL:220U21	78	E2A:1173U19	95	376 bp	
		E2A(19p13)	E2APRL:696L18	61	E2APRL:673L18	103		
(NP: 8, 33, 44, 45)	tal1[d1-3]	SIL(1p34)	SIL:24U19	52	SIL:83U20	98	183 bp	
		TAL1(1p34)	TAL1:203L21	55	TAL1:179L20	86		
	t(17;19)(q22;p13)	HLF(17q22)	E2AHLF:1685L20	62	E2AHLF:1543L20	100	390 bp	
		E2A(19p13)						
	t(12;21)(p13;q22)	TEL(12p13)	TELAML1:871U23	44	TELAML1:944U23	104	293 bp	2 variants
		AML1(21q22)	TELAML1:1336L23	37	TELAML1:1216L21	87	332 bp	
R4	t(3;21)(q26;q22)	EVI-1(3q26)	AML1EVI:1897U21	60	AML1MGT8:1896U20	128	446 bp	
		AML1(21q22)	AML1EVI:2376L24	53	AML1EVI:2345L21	97		
(NP: 11, 17, 32, 48)	t(7;10)(q35;q24)	Activation of	HOX11:590U20	67	HOX11:617U22	106	212 bp	
	t(10;14)(q24;q11)	HOX11(10q24)	HOX11:857L21	70	HOX11:810L19	113		
	t(8;21)(q22;q22)	MTG8(8q22)	AML1MGT8:2437L23	73	AML1MGT8:2226L22	102	353 bp	
		AML1(21q22)						
	t(16;21)(p11;q22)	FUS(16p11)	TLSERG:649U19	69	TLS:690U19	93	274 bp	
		ERG(21q22)	EWSERG:979L22	72	TLSERG:946L19	116		

R-mix no.	Chromosomal Rearrangement	genes	PCR primers (1st PCR)	SEQ ID NO:	PCR primers (2nd PCR)	SEQ ID NO:	PCR fragments	Comments
R5	t(4;11)(q21;q23) type A+B+C	AF4(4q21) ALL1(11q23)	ALLAF10A:3730U20	49	ALL1AF4:3751U20	114	75 bp	9 variants
			ALL1:3955U24	38	ALL1:3996U24	88	to	
(NP: 6, 12, 24, 18A, 18B)			ALL1AF4:4321L29	57	ALL1AF4:4291L25	122	321 bp	
	t(9;11)(q22;q23) type A+B	AF9(9q22) ALL1(11q23)	AF9:1498L22	77	ALL1AF9:4092L25	108	208 bp	6 variants
			ALL1AF9:4143L24	48	AF9:1466L26	105	to 368 bp	
	t(1;11)(q21;q23)	AF1q(1q21) ALL1(11q23)	ALL1AF1Q:4211L22	64	AF1Q:580L20	127	287 bp to 401 bp	3 variants
	t(11;19)(q23;p13.3)	ALL1(11q23) ENL(19p13)	ALL1ENL:4215L22	42	ALL1ENL:4164L19	112	73 bp to 187 bp	3 variants
R6	t(9;22)(q34;q11) types e1a2, b2a2,b3a2	ABL(9q34) BCR(22q11)	BCR1ABL:1698U19	54	BCR1ABL:1777U19	85	320 bp	e1a2
			BCR1ABL:2093L20	83	BCR1ABL:2074L23	84	472 bp	b3a2
(NP: 13, 19, 20, 43)			BCR2ABL:3060U23	59	BCR2ABL:3128U22	91	397 bp	b2a2
	t(9;12)(q34;p13)	ABL(9q34) TEL(12p13)	TELPDGF:309U21	63	TELPDGF:343U24	99	366 bp	
	t(5;12)(q33;p13)	PDGFβ(5q33) TEL(12p13)	TELPDGF:834L22	45	TELPDGF:642L22	94	321 bp	
R7	t(6;9)(p23;q34)	CAN(9q34) DEK(6p23)	DEKCAN:870U24	74	DEKCAN:892U21	89	320 bp	
			DEKCAN:1422L21	66	SETCAN:925L20	120		
(NP: 14, 15, 42)	?t(9;9)	CAN(9q34) SET(9q34)	SETCAN:468U23	43	SETCAN:552U24	126	393 bp	
	t(3;3)(q21;q26) inv(3;3)(q21;q26) t(2;3)(p21;q26) t(3;21)(q26;q22) t(3,5)(q26;q34)	Activation of EVI1(3q26)	AML1EVI:4331U23	47	AML1EVI:4509U21	101		
			AML1EVI:4866L21	75	AML1EVI:4746L25	123	262 bp	

R-mix no.	Chromosomal Rearrangement	genes	PCR primers (1st PCR)	SEQ ID NO:	PCR primers (2nd PCR)	SEQ ID NO:	PCR fragments	Comments
R8	t(11;17)(q23;q21) types A+B	PLZF(11q23) RARA(17q21)	PLZFRARA:1092U21 BCR3:1460L19	46 33	PLZFRARA:1252U21 BCR3:1428L22	121 96	315 bp 402 bp	
(NP: 23, 30, 31, 9, 50, 51)	t(15;17)(q21;q22) L-, V-, S-forms	PML(15q22) RARA(17q21)	BCR1:1338U19 BCR3:988U19	40 71	BCR1:1497U21 BCR3:1057U20	111 92	427 bp ±427 bp 393 bp	L-form V-form S-form
	t(2;5)(p23;q35)	ALK(2p23) NPM(5q35)	ALKNPM:200U25 ALKNPM:627L21	35 41	ALKNPM:313U21 ALKNPM:590L19	117 107	302 bp	
	t(5;17)(q35;q22) S-, L-forms	NPM(5q35) RARA(17q21)					105 bp 234 bp	S-form L-form
	t(3;5)(q25.1;q34)	MLF(3q25.1) NPM(5q34)	MLF1:235L27	38	MLF1:192:L28	133	289+333 362+406	4 splice variants
41NP	Positive control	E2A(19p13)	E2APRL:220U21 E2A:1883L22	78 82	E2A:1173U19 E2A:1844L19	103 90	694 bp	Included in all R and NP mixes

M-mix no.	PCR Primers, 1st PCR	SEQ ID NO:	PCR mix pmol/µl	PCR Primers, 2nd PCR	SEQ ID NO:	PCR mix pmol/µl
	ALLAF10A:3730U20	49	5	ALL1AF4:3750U20	139	10
	ALL1AF6:4074L21	50	5	ALL1AF6:4037L22	109	10
	ALLELL:4236L23	34	5	ALLELL:4191L22	132	10
	ALL1:3955U23	143	5	ALL1:3996U24	88	10
	AFX:812L20	135	5	AFX:697L20	140	10
M1	ALL1:351L23	136	5	ALL1:335L22	141	10
	ALL1:3181L20	137	5	ALL1:3067L21	142	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
	ALLAF10A:3730U20	49	5	ALL1AF4:3750U20	139	10
	ALL1:3955U24	36	5	ALL1:3995U22	143	10
	ALL1AF1:4048L22	65	5	ALL1AF1:3907L27	144	10
	AF17:1937L21	51	5	ALL1AF17:4032L22	145	10
M2	ALLAF10A:3968L23	56	5	ALLAF10A:3932L21	134	10
	ALLAF10B:4031L22	79	5	ALLAF10B:3997L22	146	10
	AF10:728L22	76	5	AF10:685L21	115	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
	E2APRL:696L18	61	5	E2APRL:675L19	150	10
	SIL:24U18	147	5	SIL:83U20	98	10
	TAL1:203L20	148	5	TAL1:179L20	86	10
M3	E2AHLF:1685L20	62	5	E2AHLF:1543L20	100	10
	TELAML1:871U23	44	5	TELAML1:944U23	104	10
	TELAML1:1342L23	149	5	TELAML1:1168L18	151	10
	E2A:1045U21	138	5	E2A:1173U19	103	10
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
	AML1EVI:1897U21	60	5	AML1MTG8:1895U20	128	10
	AML1EVI:2376L24	152	5	AML1EVI:2345L21	97	10
	AML1MTG8:2259L21	153	5	AML1MTG8:2226L22	102	10
	TLSERG:649U19	69	5	TLSERG:695U20	156	10
M4	EWSERG:979L22	72	5	TLSERG:945L19	116	10
	EAP:990L22	154	5	EAP:781L20	157	10
	AMLEVI:2776L22	155	5	AMLEVI:2720L22	158	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
	ALLAF10A:3730U20	49	5	ALL1AF4:3750U20	139	10
	ALL1:3955U24	36	5	ALL1:3996U23	143	10
	ALL1AF4:4393L25	159	5	ALL1AF4:4291L25	122	10
	ALL1AF9:4143L24	48	5	ALL1AF9:4092L24	108	10
M5	AF9:1498L22	77	5	AF9:1466L26	105	10
	ALL1AF1Q:4281L20	160	5	AF1Q:580L20	127	10
	ALL1ENL:4195L19	161	5	ALL1ENL:4164L19	112	10
	ENL:1321L21	162	5	ENL1256L19	165	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		

M-mix no.	PCR Primers, 1st PCR	SEQ ID NO:	PCR mix pmol/µl	PCR Primers, 2nd PCR	SEQ ID NO:	PCR mix pmol/µl
M6	BCR1ABL:1698U19	54	5	BCR1ABL:1777U19	85	10
	BCR2ABL:3060U23	59	5	BCR2ABL:3128U22	91	10
	BCR1ABL:2093L20	83	5	BCR1ABL:2074L23	84	10
	TEL:56U24	166	5	TEL:114U19	169	10
	TELPDGF:595L22	167	5	TELPDGF:555L23	170	10
	MN1:5019L25	168	5	MN1:4884L21	171	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
M7	DEKCAN:667U20	172	5	DEKCAN:892U21	89	10
	DEKCAN:1427L19	173	5	SETCAN:925L20	120	10
	SETCAN:468U22	174	5	SETCAN:552U24	126	10
	CBFBMYHC:269U20	175	5	CBFBMYHC:344U21	129	10
	CBFBMYHC:752L22	39	5	CBFBMYHC:595L19	125	10
	MYH11:1377L20	176	5	CBFBMYHA:818L21	177	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		
M8	PLZFRARA:1092U21	46	5	PLZFRARA:1252U21	121	10
	BCR1:1338U19	40	5	BCR1:1497U21	111	10
	BCR3:988U19	71	5	BCR3:1057U20	92	10
	BCR3:1460L19	33	5	BCR3:1428L22	96	10
	ALKNPM:200U25	35	5	ALKNPM:313U21	117	10
	ALKNPM:627L21	41	5	ALKNPM:590L19	107	10
	MLF1:235L27	38	5	MLF1:192L28	133	10
	E2A:1045U21	138	5	E2A:1173U19	103	7.5
	E2A:1883L22	82	5	E2A:1844L19	90	7.5
	H_2O			H_2O		

Chromosomal alteration included in the R-mix Multiplex RT-PCR analysis.

Chromosomal alteration	Genes involved	Fusion gene §	NP-mix No	PCR Mix No. ‡	Size of PCR Fragment #	Positive cell line	Presence
inv(16)(p13q22)	CBFβ (16q22)	CBFβ/MYH11 (A)	2	R1A	270	ME-1*	AML
	MYH11 (16p13)	CBFβ/MYH11 (B)	4	R1B	483		AML
		CBFβ/MYH11 (C)	4	R1B	663		AML
		CBFβ/MYH11 (D)	2	R1A	337		AML
		CBFβ/MYH11 (E)	2	R1A	544		AML
		CBFβ/MYH11 (F)	4	R1B	174		AML
		CBFβ/MYH11 (G)	4	R1B	241		AML
		CBFβ/MYH11 (H)	4	R1B	348		AML
t(X;11)(q13;q23)	MLL1 (11q23)	MLLex6/AFX	46	R1C	448		T
	AFX (Xq13)	MLLex7/AFX	46	R1C	235 (480)		T
		MLLex8/AFX	46	R1C	449 (694)	Karpas-45*	ALL
		MLLex9/AFX	46	R1C	596 (841)		T
t(6;11)(q27;q23)	MLL1 (11q23)	MLLex6/AF6	16	R1D	308	ML-2	AML, ALL†
	AF6 (6q27)	MLLex7/AF6	16	R1D	195 (440)		AML
		MLLex8/AF6	16	R1D	309 (594)		T
		MLLex9/AF6	16	R1D	456 (741)		T
t(11;19)(q23;p13.1)	MLL1 (11q23)	MLLex6/ELL	25	R1E	330		T
	ELL (19p13.1)	MLLex7/ELL	25	R1E	217 (462)		AML
		MLLex8/ELL	25	R1E	301 (576)		T
		MLLex9/ELL	25	R1E	448 (723)		T
		MLLex6/ELL-ins120	25	R1E	450		T
		MLLex7/ELL-ins120	25	R1E	337 (582)		AML
		MLLex8/ELL-ins120	25	R1E	451 (696)		AML
		MLLex9/ELL-ins120	25	R1E	598 (845)		T
t(1;11)(p32;q23)	MLL1 (11q23)	MLLex6/AF-1p	5	R2A	300		ALL
	AF-1p (1p32)	MLLex7/AF-1p	5	R2A	187 (432)		T
		MLLex8/AF-1p	5	R2A	301 (546)		T
		MLLex9/AF-1p	5	R2A	448 (693)		T
t(11;17)(q23;q21)	MLL1 (11q23)	MLLex5/AF17	22	R2B	281		AML
	AF17 (17q21)						
t(10;11)(p12;q23)	MLL1 (11q23)	MLLex5/AF10 (A:2222)	21A	R2C	202		AML
	AF10 (10p12)	MLLex6/AF10 (B:979)	21B	R2D	270		AML
		MLLex7/AF10 (B:979)	21B	R2D	157 (402)		AML
		MLLex8/AF10 (B:979)	21B	R2D	271 (516)		T
		MLLex9/AF10 (B:979)	21B	R2D	418 (663)		T
		MLLex6/AF10 (C:2110)	21A	R2C	388		AML
		MLLex7/AF10 (C:2110)	21A	R2C	275 (520)		T
		MLLex8/AF10 (C:2110)	21A	R2C	389 (634)		T
		MLLex9/AF10 (C:2110)	21A	R2C	536 (781)		T
		MLLex6/AF10 (D:883)	21B	R2D	366		AML
		MLLex7/AF10 (D:883)	21B	R2D	253 (498)		AML
		MLLex8/AF10 (D:883)	21B	R2D	367 (612)		T
		MLLex9/AF10 (D:883)	21B	R2D	514 (759)		T
		MLLex6/AF10 (E:589)	21E	R2E	267		AML
		MLLex7/AF10 (E:589)	21E	R2E	154 (399)		T

		MLLex8/AF10 (E:589)	21E	R2E	268 (513)		T
		MLLex9/AF10 (E:589)	21E	R2E	415 (660)		T
		MLLex5/AF10 (F:1931)	21A	R2C	493		AML
dupMLL (11q23)	MLL (11q23)	MLLex5/MLLex2	49	R2F	184		ALL
	MLL (11q23)	MLLex6/MLLex2	49	R2F	258		AML, ALL
		MLLex7/MLLex2	49	R2F	145 (390)		AML
		MLLex8/MLLex2	49	R2F	259 (504)		AML
		MLLex9/MLLex2	49	R2F	406 (651)		AML
t(1;19)(q23;p13)	E2A (19p13)	E2A/PBX1 (I)	8	R3A	376	697	ALL
	PBX1 (1q23)	E2A/PBX1 (Ia)	8	R3A	403		ALL
t(17;19)(q22;p13)	E2A (19p13)	E2Aex13/HLFex4 (I)	33	R3B	390	HAL-01	ALL
	HLF (17q22)	E2Aex13insHLFex4 (I)	33	R3B	417		ALL
		E2Aex12/HLFex4 (II)	33	R3B	207		ALL
t(12;21)(p13;q22)	TEL (12p13)	TEL/AML1	44	R3C	293		ALL
	AML1 (21q22)	TEL/AML1	44	R3C	332		ALL
TAL1[D]	SIL (1p34) TAL1 (1p34)	SIL/TAL1 d1+d2	45	R3D	183	RPMI8402	T-ALL
t(8;21)(q22;q22)	AML1 (21q22) MGT8 (8q22)	AML1ex5/ETO	17	R4A	353	Kasumi-1*	AML
t(3;21)(q26;q22)$	AML1 (21q22) MDS1 (3q26)	AML1ex5/MDS1/(EVI1)	11	R4B	446	SKH1*	CML-BC, AML, MDS
	(EVI1) (3q26)	AML1ex6/MDS1/(EVI1)	11	R4B	638		CML-BC, AML, MDS
t(16;21)(p11;q22)	TLS (16p11)	TLS/ERG (a)	32	R4C	318	UTP-L12*	AML
	ERG (21q22)	TLS/ERG (b)	32	R4C	274	UTP-L12*	AML
		TLS/ERG (c)	32	R4C	239	UTP-L12*	AML, ALL†
		TLS/ERG (d)	32	R4C	344		ALL
		TLS/ERG (e)	32	R4C	413		ALL
t(7;10)(q35;q32) t(10;14)(q24;q11)	Activation of HOX11 (10q32)	HOX11o	48	R4D	212	RPMI8402	T-ALL, AML, ALL, CML
t(1;11)(q21;q23)	MLL1 (11q23)	MLL1ex6/AF1q	6	R5E	300		AMMOL
	AF1q (1q21)	MLL1ex7/AF1q	6	R5E	187		T
		MLL1ex8/AF1q	6	R5E	301 (546)		T
		MLL1ex9/AF1q	6	R5E	448 (693)		T
t(4;11)(q21;q23)	MLL1 (11q23)	MLLex6/AF4 (a:1414)	12	R5A	317		ALL
	AF4 (4q21)	MLLex7/AF4 (a:1414)	12	R5A	204 (449)	RS4;11	ALL
		MLLex8/AF4 (a:1414)	12	R5A	318 (563)		ALL
		MLLex9/AF4 (a:1414)	12	R5A	465 (710)		T
		MLLex6/AF4 (b:1459)	12	R5A	272	MV4;11	ALL
		MLLex7/AF4 (b:1459)	12	R5A	159 (404)		ALL
		MLLex8/AF4 (b:1459)	12	R5A	273 (518)		ALL
		MLLex9/AF4 (b:1459)	12	R5A	420 (665)		T
		MLLex6/AF4 (c:1546)	12	R5A	185		T
		MLLex7/AF4 (c:1546)	12	R5A	72 (317)		ALL
		MLLex8/AF4 (c:1546)	12	R5A	186 (431)		ALL

		MLLex9/AF4 (c:1546)	12	R5A	333 (578)		T
t(11;19)(q23;p13.3)	MLL (11q23) ENL (19p13.3)	MLLex6/ENL (A:177)	24	R5B	186		ALL
		MLLex7/ENL (A:177)	24	R5B	73 (318)	KOCL-44*	ALL
		MLLex8/ENL (A:177)	24	R5B	187 (432)	KOCL-44*	ALL
		MLLex9/ENL (A:177)	24	R5B	334 (579)		T
t(9;11)(q22;q23)	MLL (11q23) AF9 (9q22)	MLLex6/AF9 (A)	18A	R5C	321		AML
		MLLex7/AF9 (A)	18A	R5C	208 (453)	Mono-Mac-6	AML
		MLLex8/AF9 (A)	18A	R5C	322 (567)	Mono-Mac-6	AML
		MLLex9/AF9 (A)	18A	R5C	469 (714)		T
		MLLex6/AF9 (B)	18B	R5D	367		AML
		MLLex7/AF9 (B)	18B	R5D	254 (499)		T
		MLLex8/AF9 (B)	18B	R5D	368 (613)		T
		MLLex9/AF9 (B)	18B	R5D	515 (760)		T
t(9;22)(q34;q11)	BCR (22q11) ABL (9q34)	BCR/ABL e1a2	19	R6A	320		ALL
		BCR/ABL b2a2	20	R6B	397		CML
		BCR/ABL b3a2	20	R6B	472		CML
t(9;12)(q34;p13)	TEL (12p13) ABL (9q34)	TEL/ABL	43	R6C	366		ALL
t(5;12)(q33;p13)	TEL (12p13) PDGFRβ (5q33)	TEL/PDGFRβ	13	R6D	321		CMML, MDS
t(6;9)(q23;q34)	DEK (6q23) CAN (9q34)	DEK/CAN	14	R7A	320		AML
?t(9;9)	SET (9q34) CAN (9q34)	SET/CAN	15	R7B	393		AUL
t(3;3)(q21;q26)	Activation of EVI1	EVI1o	42	R7C	262	JOSK-I	AML
inv(3)(q21q26)			42	R7C	262		CML-BC, AML
ins(3)(q21q25q26)			42	R7C	262		AML
t(3;4)(q26;q21)			42	R7C	262		MDS, AML
t(3;12;20)(q26;q12;q13)			42	R7C	262		CML-BC
t(3;21)(q26;q22)$	AML1 (21q22) EVI1 (3q26)	AML1ex5/(MDS1)/EVI1	42	R7C	262	SKH1*	CML-BC, AML, MDS
		AML1ex5/EVI1	42	R7C	262		

t(11;17)(q23;q21)	PLZF (11q23) RARAα (17q21)	PLZF/RARα (A:1365) PLZF/RARα (B:1452)	23 23	R8A R8A	315 402		APL APL
t(15;17)(q21;q22)	PML (15q21) RARα (17q21)	PMLex3/RARαex2 S-form (=BCR3)	30	R8C	393	NB4	APL
		PMLex3Δ/RARαex2 S-form splice variant	30	R8C	338	NB4	APL
		PMLex6/RARαex2 L-form (=BCR1)	31	R8B	427	NB4	APL
		PMLex3Δex5+6/ RARαex2, L-form splice variant	30	R8C	464	NB4	APL
		PMLΔex6-(+/-)ins- RARαex2 V-form (=BCR2)	31	R8B	+/-427		APL
t(2;5)(p23;q35)	NPM (5q35) ALK (2p23)	NPM/ALK	9	R8D	302	Karpas-299	ALCL, T-/ B-cell lymphomas
t(5;17)(q35;q22)	NPM (5q35) RARα (17q21)	NPM(S)/RARα NPM(L)/RARα	51 51	R8E R8E	105 234		APL APL
t(3;5)(q25.1;q34)	NPM (5q35) MLF1 (3q25.1)	NPM/MLF1	50	R8F	289		MDS, AML

Abbreviations: No, number; t, translocation; inv, inversion; p, short chromosome arm; q, long chromosome arm; ex, exon; ins, insertion; T, theoretically possible translocation variant; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML (-BC), chronic myeloid leukemia (in blast crisis); MDS, myelodysplastic syndrome; APL, acute promyelocytic leukemia; AUL, acute undifferentiated leukemia; CMML, chronic myelomonocytic leukemia; ALCL, anaplastic large cell lymphoma; AMMOL, acute myelomonocytic leukemia.

§ Letters and numbers in parentheses after the fusion-gene indicates alternative breakpoints and/or splice variants.

‡ R1-R8, indicate multiplex reaction number, suffix A to F indicate split-out reaction.

* Cell line not available for testing described as positive: ME-1,, Karpas-45, Kasumi-1, SKH1, UTP-L12, KOCL-44.

Numbers in parentheses indicates the size of the co-amplified PCR fragment resulting from the MLL1 exon 5 primer.

¤ No fusion mRNA is generated, only expression of the gene is tested for.

$ The t(3;21)(q26;q22) resulting in a AML1/MDS/EVI1 fusion will be detected in multiplex reaction R4 and R7.

Chromosomal alteration included in the M -mix Multiplex RT-PCR analysis.

Chromosomal alteration	Genes involved	Fusion gene §	M-NP mix No	PCR Mix No. ‡	Size of PCR Fragment #	Positive cell line	Presence
dupMLL (11q23)	MLL (11q23)	MLLex5/MLLex2	M49	M1A	186		ALL
	MLL (11q23)	MLLex6/MLLex2	M49	M1A	260		AML, ALL
		MLLex7/MLLex2	M49	M1A	147 (392)		AML
		MLLex8/MLLex2	M49	M1A	261 (506)		AML
		MLLex9/MLLex2	M49	M1A	408 (653)		AML
dupMLL (11q23)	MLL (11q23)	MLLex5/MLLex4	M101	M1B	193		T
	MLL (11q23)	MLLex6/MLLex4	M101	M1B	267		AML
		MLLex7/MLLex4	M101	M1B	154 (399)		T
		MLLex8/MLLex4	M101	M1B	268 (513)		T
		MLLex9/MLLex4	M101	M1B	415 (660)		T
t(X;11)(q13;q23)	MLL1 (11q23)	MLLex6/AFX	M46	M1C	245		T
	AFX (Xq13)	MLLex7/AFX	M46	M1C	132 (377)		T
		MLLex8/AFX	M46	M1C	246 (491)	Karpas-45*	ALL
		MLLex9/AFX	M46	M1C	393 (638)		T
t(6;11)(q27;q23)	MLL1 (11q23)	MLLex6/AF6	M16	M1D	309	ML-2	AML, ALL†
	AF6 (6q27)	MLLex7/AF6	M16	M1D	195 (441)		AML
		MLLex8/AF6	M16	M1D	309 (595)		T
		MLLex9/AF6	M16	M1D	456 (742)		T
t(11;19)(q23;p13.1)	MLL1 (11q23)	MLLex6/ELL	M25	M1E	331		T
	ELL (19p13.1)	MLLex7/ELL	M25	M1E	217 (463)		AML
		MLLex8/ELL	M25	M1E	301 (577)		T
		MLLex9/ELL	M25	M1E	448 (724)		T
		MLLex6/ELL-ins120	M25	M1E	451		T
		MLLex7/ELL-ins120	M25	M1E	337 (583)		AML
		MLLex8/ELL-ins120	M25	M1E	451 (697)		AML
		MLLex9/ELL-ins120	M25	M1E	598 (846)		T
t(1;11)(p32;q23)	MLL1 (11q23)	MLLex6/AF-1p	M5	M2A	183		ALL
	AF-1p (1p32)	MLLex7/AF-1p	M5	M2A	70 (315)		T
		MLLex8/AF-1p	M5	M2A	184 (429)		T
		MLLex9/AF-1p	M5	M2A	331 (576)		T
t(11;17)(q23;q21)	MLL1 (11q23)	MLLex5/AF17	M22	M2B	282		AML
	AF17 (17q21)						
t(10;11)(p12;q23)	MLL1 (11q23)	MLLex5/AF10 (A:2222)	M21A	M2C	203		AML
	AF10 (10p12)	MLLex6/AF10 (B:979)	M21B	M2D	271		AML
		MLLex7/AF10 (B:979)	M21B	M2D	158 (403)		AML
		MLLex8/AF10 (B:979)	M21B	M2D	272 (517)		T
		MLLex9/AF10 (B:979)	M21B	M2D	419 (664)		T
		MLLex6/AF10 (C:2110)	M21A	M2C	389		AML
		MLLex7/AF10 (C:2110)	M21A	M2C	276 (521)		T
		MLLex8/AF10 (C:2110)	M21A	M2C	390 (635)		T
		MLLex9/AF10 (C:2110)	M21A	M2C	537 (782)		T
		MLLex6/AF10 (D:883)	M21B	M2D	367		AML
		MLLex7/AF10 (D:883)	M21B	M2D	254 (499)		AML
		MLLex8/AF10 (D:883)	M21B	M2D	368 (613)		T

		MLLex9/AF10 (D:883)	M21B	M2D	515 (760)		T
		MLLex6/AF10 (E:589)	M21E	M2E	268		AML
		MLLex7/AF10 (E:589)	M21E	M2E	155 (400)		T
		MLLex8/AF10 (E:589)	M21E	M2E	269 (514)		T
		MLLex9/AF10 (E:589)	M21E	M2E	415 (661)		T
		MLLex5/AF10 (F:1931)	M21A	M2C	494		AML
t(1;19)(q23;p13)	E2A (19p13)	E2A/PBX1 (I)	M8	M3A	376	697	ALL
	PBX1 (1q23)	E2A/PBX1 (Ia)	M8	M3A	403		ALL
t(17;19)(q22;p13)	E2A (19p13)	E2Aex13/HLFex4 (I)	M33	M3B	390	HAL-01	ALL
	HLF (17q22)	E2Aex13insHLFex4 (I)	M33	M3B	417		ALL
		E2Aex12/HLFex4 (II)	M33	M3B	207		ALL
t(12;21)(p13;q22)	TEL (12p13)	TEL/AML1	M44	M3C	242		ALL
	AML1 (21q22)	TEL/AML1	M44	M3C	281		ALL
TAL1[D]	SIL (1p34) TAL1 (1p34)	SIL/TAL1 d1+d2	M45	M3D	183	RPMI8402	T-ALL
t(8;21)(q22;q22)	AML1 (21q22) MGT8 (8q22)	AML1ex5/ETO	M17	M4A	353	Kasumi-1*	AML
t(3;21)(q26;q22)	AML1 (21q22) MDS1 (3q26)	AML1ex5/MDS1/(EVI1)	M11	M4B	446	SKH1*	CML-BC, AML, MDS
	(EVI1) (3q26)	AML1ex6/MDS1/(EVI1)	M11	M4B	638		CML-BC, AML, MDS
t(3;21)(q26;q22)	AML1 (21q22)	AML1ex5/EVI1ex2	M102	M4C	540		AML
	EVI1 (3q26)	AML1ex5/EVI1ex3	M102	M4C	350		AML
t(3;21)(q26;q22)	AML1 (21q22)	AML1ex5/EAP	M103	M4D	475		AML
	EAP (3q26)	AML1ex6/EAP	M103	M4D	355		AML
t(16;21)(p11;q22)	TLS (16p11)	TLS/ERG (a)	M32	M4E	313	UTP-L12*	AML
	ERG (21q22)	TLS/ERG (b)	M32	M4E	269	UTP-L12*	AML
		TLS/ERG (c)	M32	M4E	234	UTP-L12*	AML, ALL
		TLS/ERG (d)	M32	M4E	339		ALL
		TLS/ERG (e)	M32	M4E	408		ALL
t(4;11)(q21;q23)	MLL1 (11q23)	MLLex6/AF4 (a:1414)	M12	M5A	318		ALL
	AF4 (4q21)	MLLex7/AF4 (a:1414)	M12	M5A	204 (450)	RS4;11	ALL
		MLLex8/AF4 (a:1414)	M12	M5A	318 (564)		ALL
		MLLex9/AF4 (a:1414)	M12	M5A	465 (711)		T
		MLLex6/AF4 (b:1459)	M12	M5A	273	MV4;11	ALL
		MLLex7/AF4 (b:1459)	M12	M5A	159 (405)		ALL
		MLLex8/AF4 (b:1459)	M12	M5A	273 (519)		ALL
		MLLex9/AF4 (b:1459)	M12	M5A	420 (666)		T
		MLLex6/AF4 (c:1546)	M12	M5A	186		T
		MLLex7/AF4 (c:1546)	M12	M5A	72 (318)		ALL
		MLLex8/AF4 (c:1546)	M12	M5A	186 (432)		ALL
		MLLex9/AF4 (c:1546)	M12	M5A	333 (579)		T
t(11;19)(q23;p13.3)	MLL (11q23)	MLLex6/ENL (A:177)	M24	M5B	187		ALL
	ENL (19p13.3)	MLLex7/ENL (A:177)	M24	M5B	73 (319)	KOCL-44*	ALL

		MLLex8/ENL (A:177)	M24	M5B	187 (433)	KOCL-44*	ALL
		MLLex9/ENL (A:177)	M24	M5B	334 (580)		T
t(11;19)(q23;p13.3)	MLL (11q23)	MLLex6/ENL (B:)	M24	M5C	315	KOPN-1*	ALL
	ENL (19p13.3)	MLLex7/ENL (B:)	M24	M5C	201 (447)		T
		MLLex8/ENL (B:)	M24	M5C	316 (561)		T
		MLLex9/ENL (B:)	M24	M5C	463 (708)		T
t(9;11)(q22;q23)	MLL (11q23)	MLLex6/AF9 (A)	M18A	M5D	322		AML
	AF9 (9q22)	MLLex7/AF9 (A)	M18A	M5D	208 (454)	Mono-Mac-6	AML
		MLLex8/AF9 (A)	M18A	M5D	322 (568)	Mono-Mac-6	AML
		MLLex9/AF9 (A)	M18A	M5D	469 (715)		T
		MLLex6/AF9 (B)	M18B	M5E	368		AML
		MLLex7/AF9 (B)	M18B	M5E	254 (500)		T
		MLLex8/AF9 (B)	M18B	M5E	368 (614)		T
		MLLex9/AF9 (B)	M18B	M5E	515 (761)		T
t(1;11)(q21;q23)	MLL1 (11q23)	MLL1ex6/AF1q	M6	M5F	301		AMMOL
	AF1q (1q21)	MLL1ex7/AF1q	M6	M5F	187 (426)		T
		MLL1ex8/AF1q	M6	M5F	301 (547)		T
		MLL1ex9/AF1q	M6	M5F	448 (694)		T
t(9;22)(q34;q11)	BCR (22q11)	BCR/ABL e1a2	19	M6A	320		ALL
	ABL (9q34)	BCR/ABL b2a2	20	M6B	397		CML
		BCR/ABL b3a2	20	M6B	472		CML
t(9;12)(q34;p13)	TEL (12p13)	TEL/ABL	M43	M6C	595		ALL
	ABL (9q34)						
t(5;12)(q33;p13)	TEL (12p13)	TEL/PDGFRβ	M13	M6D	472		CMML, MDS
	PDGFRβ (5q33)						
t(12;22)(p13;q11)	TEL (12p13)	TEL/MN1	M105	M6E	244		AML
	MN1(22q11)		M105	M6E	409		AML
t(6;9)(q23;q34)	DEK (6q23)	DEK/CAN	M14	M7A	320		AML
	CAN (9q34)						
?t(9;9)	SET (9q34)	SET/CAN	M15	M7B	393		AUL
	CAN (9q34)						
inv(16)(p13q22)	CBFβ (16q22)	CBFβ/MYH11 (A)	M2	M7C	270	ME-1*	AML
	MYH11 (16p13)	CBFβ/MYH11 (B)	M4	M7D	434		AML
		CBFβ/MYH11 (C)	M4	M7D	614		AML
		CBFβ/MYH11 (D)	M2	M7C	337		AML
		CBFβ/MYH11 (E)	M2	M7C	544		AML
		CBFβ/MYH11 (F)	M4	M7D	125		AML
		CBFβ/MYH11 (G)	M4	M7D	192		AML
		CBFβ/MYH11 (H)	M4	M7D	299		AML

t(11;17)(q23;q21)	PLZF (11q23)	PLZF/RARα (A:1365)	23	M8A	315		APL
	RARAα (17q21)	PLZF/RARα (B:1452)	23	M8A	402		APL
t(15;17)(q21;q22)	PML (15q21) RARα (17q21)	PMLex3/RARαex2 S-form (=BCR3)	30	M8C	393	NB4	APL
		PMLex3Δ/RARαex2 S-form splice variant	30	M8C	338	NB4	APL
		PMLex6/RARαex2 L-form (=BCR1)	31	M8B	427	NB4	APL
		PMLex3Δex5+6/ RARαex2, L-form splice variant	30	M8C	464	NB4	APL
		PMLΔex6-(+/-)ins-RARαex2 V-form (=BCR2)	31	M8B	+/-427		APL
t(2;5)(p23;q35)	NPM (5q35) ALK (2p23)	NPM/ALK	9	M8D	302	Karpas-299	ALCL, T-/ B-cell lymphomas
t(5;17)(q35;q22)	NPM (5q35)	NPM(S)/RARα	51	M8E	105		APL
	RARα (17q21)	NPM(L)/RARα	51	M8E	234		APL
t(3;5)(q25.1;q34)	NPM (5q35) MLF1 (3q25.1)	NPM/MLF1	50	M8F	289		MDS, AML

Abbreviations: No, number; t, translocation; inv, inversion; p, short chromosome arm; q, long chromosome arm; ex, exon; ins, insertion; T, theoretically possible translocation variant; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML (-BC), chronic myeloid leukemia (in blast crisis); MDS, myelodysplastic syndrome; APL, acute promyelocytic leukemia; AUL, acute undifferentiated leukemia; CMML, chronic myelomonocytic leukemia; ALCL, anaplastic large cell lymphoma; AMMOL, acute myelomonocytic leukemia.

§ Letters and numbers in parentheses after the fusion-gene indicates alternative breakpoints and/or splice variants.

‡ R1-R8, indicate multiplex reaction number, suffix A to F indicate split-out reaction.

* Cell line not available for testing described as positive: ME-1,, Karpas-45, Kasumi-1, SKH1, UTP-L12, KOCL-44, KOPN-1.

Numbers in parentheses indicates the size of the co-amplified PCR fragment resulting from the MLL1 exon 5 primer.

Nested RT-PCR Primers

M-NP-mix ver.1

M-NP mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID No:	PCR-Primers N2 (2nd PCR)	SEQ ID No:.	Pos. Controle
M2	inv(16)(p13;q22)	CBFβ (16q22) MYH11(16p13)	CBFBMYHC:269U20 CBFBMYHC:752L22	175 39	CBFBMYH:344U21 CBFBMYHC:595L19	129 125	Pt:
M4	inv(16)(p13;q22)	CBFβ (16q22) MYH11(16p13)	CBFBMYHC:269U20 MYH11:1377L20	175 176	CBFBMYH:344U21 CBFBMYHA:818L21	129 177	No
M45	tal1^{d1-3} (40 kb deletion)	SIL1(1p34) TAL1(1p34)	SIL:24U18 TAL1:203L20	147 148	SIL:83U20 TAL1:179L20	98 86	RPMI
M49	dup(11q23) dup exon 2-5/9	ALL1(11q23)	ALLAF10A:3730U20 ALL13955U23 ALL1:351L23	49 36 136	ALL1AF4:3750U20 ALL1:3996U24 ALL1:335L22	139 88 141	Pt
M101	dup(11q23) dup exon 4-5/9	ALL1(11q23)	ALLAF10A:3730U20 ALL13955U23 ALL1:3181L20	49 36 137	ALL1AF4:3750U20 ALL1:3996U24 ALL1:3067L21	139 88 142	No
M46	t(X;11)(q13;q23)	AFX1(X;q13) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AFX:812L20	49 36 135	ALL1AF4:3751U20 ALL1:3996U24 AFX:697L20	139 88 140	Cell line: Karpas 45
M5	t(1;11)(p32;q23)	AF1p(1p32) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF1:4048L22	49 36 65	ALL1AF4:3750U20 ALL1:3995U22 ALL1AF1:3907L27	139 143 144	No

M-NP mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID No:	PCR-Primers N2 (2nd PCR)	SEQ ID No:.	Pos. Controle
M6	t(1;11)(q21;q23)	AF1q(1q21) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF1Q:4281L20	49 36 160	ALL1AF4:3750U20 ALL1:3996U23 AF1Q:580L20	139 164 127	No
M8	t(1;19)(q23;p13)	PBX1(1q23) E2A(19p13)	E2A:1045U21 E2APRL:696L18	138 61	E2A:1173U19 E2APRL:675L19	103 150	Pt+Cell line: 697
9	t(2;5)(p23;q35)	ALK(2p23) NPM(5q35)	ALKNPM:200U25 ALKNPM:627L21	35 41	ALKNPM:313U21 ALKNPM:590L19	117 107	Pt+ Karpas 299
50	t(3;5)(q25.1;q34)	MLF(3q25.1) NPM(5q34)	ALKNPM:200U25 MLF1:235L27	35 38	ALKNPM:313U21 MLF1:192:L28	117 133	No
M11	t(3;21)(q26;q22)	MDS(3q26) AML1(21q22)	AML1EVI:1897U21 AML1EVI:2376L24	60	AML1EVI:2345L21	128 97	Cell line: SKHI
M102	t(3;21)(q26;q22)	EVI-1(3q26) AML1(21q22)	AML1EVI:1897U21 AML1EVI:2776L22	60 155	AML1MGT8:1895U20 AML1EVI:2720L22	128 158	
M103	t(3;21)(q26;q22)	EAP(3q26) AML1(21q22)	AML1EVI:1897U21 EAP:990L22	60 154	AML1MGT8:1895U20 EAP:781L20	128 157	
M12	t(4;11)(q21;q23)	AF4(4q21) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF4:4393L25	49 36 159	ALL1AF4:3750U20 ALL1:3996U23 ALL1AF4:4291L25	139 164 122	Cell line: RS4;11 MV4;11
M13	t(5;12)(q33;p13)	PDGFβ(5q33) TEL(12p13)	TEL:56U24 TELPDGF:595L22	166 167	TEL:114U19 TELPDGF:555L23	169 170	
51	t(5;17)(q35;q22) S-, L-forms	NPM(5q35) RARA(17q21)	NPMALK:200U25 BCR3:1460L19	35 33	NPMALK:313U21 BCR3:1428L22	117 96	

M-NP mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID No:	PCR-Primers N2 (2nd PCR)	SEQ ID No:.	Pos. Controle
M14	t(6;9)(p23;q34)	CAN(6p23) DEK(9q34)	DEKCAN:667U20 DEKCAN:1427L19	172 173	DEKCAN:892U21 SETCAN:925L20	89 120	Pt:
M15	?t(9;9)	CAN(6p23) SET(6p23)	SETCAN:468U22 DEKCAN:1427L19	174 173	SETCAN:552U24 SETCAN:925L20	126 120	No
M16	t(6;11)(q27;q23)	AF6(6q27) ALL(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF6:4074L21	49 36 50	ALL1AF4:3750U20 ALL1:3996U24 ALL1AF6:4037I22	139 88 109	Pt.+Cell line: ML-2
M17	t(8;21)(q22;q22)	MTG8(8q22) AML1(21q22)	AML1EVI:1897U21 AML1MGT8:2259-L21	60 153	AML1MGT8:1895U20 AML1MGT8:2226L22	128 102	Pt+Cell line: Kasumi-1
M18A	t(9;11)(q22;q23)	AF9(9q22) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AF9:1498L22	49 36 77	ALL1AF4:3750U20 ALL1:3996U23 AF9:1466L26	139 164 105	Cell line: Mono Mac 6
M18B	t(9;11)(q22;q23)	AF9(9q22) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 ALL1AF9:4143L24	49 36 48	ALL1AF4:3750U20 ALL1:3996U23 ALL1AF9:4092L25	139 143 108	Cell line: Mono Mac 6
M43	t(9;12)(q34;p13)	ABL(9q34) TEL(12p13)	TEL:56U24 BCR1ABL:2093L20	166 83	TEL:114U19 BCR1ABL:2074L23	169 84	No
19	t(9;22)(q34;q11) type e1a2	ABL(9q34) BCR(22q11)	BCR1ABL:1698U19 BCR1ABL:2093L20	54 83	BCR1ABL:1777U19 BCR1ABL:2074L23	85 84	Pt:
20	t(9;22)(q34;q11) type b2a2+b3a2	ABL(9q34) BCR(22q11)	BCR2ABL:3060U23 BCR1ABL:2093L20	59 83	BCR2ABL:3128U22 BCR1ABL:2074L23	91 84	Pt: Pt:

M-NP mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID No:	PCR-Primers N2 (2nd PCR)	SEQ ID No:.	Pos. Controle
M21A	t(10;11)(p14;q23) A+C type	AF10(10p14) ALL1(11q23)	ALLAF10:3730U20 ALL1:3955U24 ALLAF10A:3968L23	49 36 56	ALLAF4:3750U20 ALL1:3995U22 ALLAF10A:3932L21	139 143 134	Pt.
M21B	t(10;11)(p14;q23) B+D type	AF10(10p14) ALL1(11q23)	ALLAF10:3730U20 ALL1:3955U24 ALLAF10B:4031L22	49 36 79	ALLAF4:3750U20 ALL1:3995U22 ALLAF10B:3997L22	139 143 146	Pt.
M21E	t(10;11)(p14;q23) E type	AF10(10p14) ALL1(11q23)	ALLAF10A:3730U20 ALL1:3955U24 AF10:728L22	49 36 76	ALL1AF4:3750U20 ALL1:3995U22 AF10:685L21	139 143 115	No
M22	t(11;17)(q23;q21) A	ALL1(11q23) AF-17(17q21)	ALLAF10A:3730U20 ALL1:3955U24 AF17:1937L22	49 36 51	ALL1AF4:3750U20 ALL1:3995U22 ALL1AF17:4032L22	139 143 145	No
23	t(11;17)(q23;q21) B	PLZF(11q23) RARA(17q21)	PLZFRARA:1092U21 BCR3:1460L20	46 33	PLZFRARA:1252U21 BCR3:1428L22	121 96	No
M24	t(11;19)(q23;p13.3) A	ALL1(11q23) ENL(19p13)	ALLAF10A:3730U20 ALL1:3955U24 ALL1ENL:4195L19	49 36 161	ALL1AF4:3750U20 ALL1:3996U23 ALL1ENL:4164L19	139 164 112	Pt.. (HB1119+ KOCL33 ect.)
M104	t(11;19)(q23;p13.3) B	ALL1(11q23) ENL(19p13)	ALLAF10A:3730U20 ALL1:3955U24 ENL:1321L21	49 36 162	ALL1AF4:3750U20 ALL1:3996U23 ENL:1256L19	139 164 165	
M25	t(11;19)(q23;p13.1)	ALL1(11q23) ELL(19p13)	ALLAF10A:3730U20 ALL1:3955U24 ALLELL:4236L23	49 36 34	ALL1AF4:3750U20 ALL1:3996U24 ALLELL:4191L22	139 88 132	Pt.

M-NP mix no.	Rearrangement	Genes	PCR-Primers N1 (1st PCR)	SEQ ID No:	PCR-Primers N2 (2nd PCR)	SEQ ID No:.	Pos. Controle
M44	t(12;21)(p13;q22)	TEL(12p13) AML1(21q22)	TELAML1:871U23 TELAML1:1342L23	44 149	TELAML1:944U23 TELAML1:1168L18	104 151	Pt.
M105	t(12;22)(p13;q11)	TEL(12p13) MN1(22;q11)	TEL:56U24 MN1:5019L25	166 168	TEL:114U19 MN14884L21	169	
30	t(15;17)(q21;q22) type V+L	PML(15q22) RARA(17q21)	BCR1:1338U19 BCR3:1460L19	40 33	BCR1:1497U21 BCR3:1428L22	111171 96	Pt+Cell line: NB4
31	t(15;17)(q21;q22) type S	PML(15q22) RARA(17q21)	BCR3:988U19 BCR3:1460L19	71 33	BCR3:1057U20 BCR3:1428L22	92 96	Pt:
M32	t(16;21)(p11;q22)	FUS(16p11) ERG(21q22)	TLSERG:649U19 EWSERG:979L22	69 72	TLS:695U20 TLSERG:945L19	156 116	Pt + (UTP-L12)
M33	t(17;19)(q22;p13)	HLF(17q22) E2A(19p13)	E2A:1045U21 E2AHLF:1685L20	138 62	E2A:1173U19 E2AHLF:1543L20	103 100	Cell line: HAL-01
M41	Positive controle	E2A(19p13)	E2A:1045U21 E2A:1883L22	138 82	E2A:1173U19 E2A:1844L19	103 90	All Pts. and cell lines

In the following is given the precise details concerning the protocols used for cDNA production and PCR amplification reactions:

cDNA syntesis (in PCR-Lab #1):

1. Mix 1 µg totale RNA resuspended in 10 µl DEP H_2O with 2.5 µl 1 pmol/µl (of each) specific cDNA primer.
2. Incubate 5 min at 65°C, place on ice.
3. Add 12.5 µl McDNA-mix, mix, spin briefly. McDNA-mix (12.5 µl):
   5 µl 5X first strand buffer,
   2.5 µl 100 mM DTT,
   2.5 µl 10 mM (each) dNTP (Pharmacia),
   2.0 µl MoMLV RT 200 u/µl (BRL), and
   0.5 µl 25 u/µl RNase inhibitor (Boehringer).
4. Incubate 45 min at 37°C.
5. Dilute cDNA to 55 µl with ddH_2O.

1. PCR amplification (in PCR-Lab #1):

1. To eight 200 µl PCR tubes on ice, add 20 µl of R1A-R8A PCR-mix.
   8x RnA PCR-mix made on ice (20 µl each):
   2.5 µl 10X Taq buffer,
   0.5 µl 10 mM (each) dNTP,
   1 µl primer-mix (either R1A to R8A primer mix),
   15.7 µl ddH_2O, and
   0.3 µl 5u/µl AmpliTaq Gold (Perkin Elmer).
2. Add 5 µl diluted cDNA to each of the 8 R1A-R8A mixtures using a Biohit Proline electric dispenser or equivalent.
3. Transfer tubes to a 9600 termocycler (Perkin Elmer) and run the RA PCR:
   95°C 15 min. followed by 25 cycles:
   95°C for 30 sec.
   58°C for 30 sec.

72°C for 90 sec.

2. PCR amplification (in PCR-Lab #2):

1. Withdraw 1 µl from each of the 8 R1A-R8A PCR reactions using a 8 chanel Biohit 0.2-10 µl electric pipette and add it to 8 tubes each with 24 µl R1B-R8B PCR mix.
   8x RnB PCR-mix made on ice (24 µl each):
   2.5 µl 10X Taq buffer,
   0.5 µl 10 mM (each) dNTP,
   1 µl primer-mix (R1B-R8B),
   19.7 µl ddH_2O, and
   0.3 µl 5u/µl AmpliTaq Gold (Perkin Elmer).
2. Transfer tubes to termocycler and start RB program:
   95°C 15 min. followed by 20 cycles:
   95°C for 30 sec.
   58°C for 30 sec.
   72°C for 90 sec.,
   followed by 10 min at 72°C.

D. Agarose gel electrophoresis (in PCR Lab #3):

1. Withdraw 12.5 µl PCR product, add 4 µl 5X loading buffer and run 15 µl on a 1.5% agarose gel with 25 µl/liter 10 mg/ml EtBr for 60 min at 100 V.

Exemplary results of the inventive methods appear from Figs. 1-3. When a patient serum is negative for the chromosomal abnormalities which can be detected with a given mixture of primers, the only band visible in the gelelectrophoresis is the internal positive control (cf. e.g. lanes 1-3 and 5-8 in Fig. 1). A positive sample will manifest itself as a band deviating from the position of the internal standard (cf. lane 4 in Fig. 1). The precise location in the gel can then identify precisely the kind of rearrangement (in this case t(8;21) which results in the positive reaction. Absence of

the internal positive control indicates that the method should be repeated since false negatives might be present.

Figs. 2A and B shows essentially the same picture as Fig. 1, although for different chromosomal rearrangements; the gene identified is indicated over each panel.

In cases where it is impossible to determine the precise nature of the genetic rearrangement in a positive sample, the sample is subjected to individual (non-multiplex) PCR reactions using specific primers selected from the NP mixes listed above. Thereby, the precise variant of the chromosomal rearrangement can be determined (cf. Fig. 4).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PALLISGAARD, Niels
(B) STREET: Fasanvej 28
(C) CITY: Aarhus V.
(E) COUNTRY: Denmark
(F) POSTAL CODE (ZIP): 8210

(ii) TITLE OF INVENTION: Improved detection of fusion genes

(iii) NUMBER OF SEQUENCES: 147

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AGCTGCTTGA TG 12

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CTGCTGGGTG AG 12

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGAGCTC AG 12

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

AGGGCTTTTG AG 12

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCCTCCAGAA G 11

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CAGCGAACAA TG 12

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCCATCCATA AC 12

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTCCTTGCTG AG 12

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTGCAGGAAG GT 12

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTGGCTGGTA CT 12

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCGATCATCT TT 12

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTGCGAACTC TT 12

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CTGCCATCAC TT 12

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GCATCCAGTT GT 12

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GCTGCCATTG AT 12

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CCACTGCCTC TC 12

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ACCTGAGCTG TG 12

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GTAGCCACAG TAT 13

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GACACCGGAA G 11

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CGGTCGTTTC TC 12

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TCTCCACGAA GT 12

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CCAGCCTTGA TG 12

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TGTAGGCGTA GC 12

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGCCTCATAC TT 12

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GCTTCGCTCA G 11

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TAAGGCTGCT CT 12

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CGGTAGCATT TC 12

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CCGTGCCTCT A 11

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

AAGTGCCAAC AG 12

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TGCTGCCTCT C 11

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

TTTGGTCTCT GAT 13

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TGGTCTGGAA AG 12

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

AAGCCCTTGC AGCCCTCAC 19

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

ACACCGTGAT CTTGTCCTGT ATG 23

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

ACGAAGGCAG TCCAATTAAA GTAAC 25

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

AGCACTCTCT CCAATGGCAA TAGT 24

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

AGCCGAGTAG TTTTCATCAT TGC 23

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

AGCTCTCCCT CTACCATCAG AGATACT 27

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

AGGTCCCCTT CCAGCTTCTT CT 22

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CAAGAAAGCC AGCCCAGAG 19

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CACACTTCAG GCAGCGTCTT C 21

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CACCATCCAG TCGTGAGTGA AC 22

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

CACCGAAATC AAATGGAAAT CTG 23

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

CACTCCGTGG ATTTCAAACA GTC 23

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

CATAAGGGCT TGCTTCTCAC TG 22

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

CCACAAGGCT GACGCTGTAT T 21

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CCACTAAGCG AAAGGATGAG AAG 23

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CCAGATGTTT CCAGGTAACT CTGT 24

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CCGCCTCAGC CACCTACTAC 20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

CCGCTGACAT GCACTTCATA G 21

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

CCTCCAGGTC TGGCTCTGTG T 21

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

CGACCCCAAC GTCCCAGAG 19

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CGATCTTCCT TTTGGTCCAT ATTC 24

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

CGCTCTCCCT CGCAGAACT 19

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

CGGTCATCCT GGGGCATATT T 21

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

CTGTTCTATG CTGGCTGCTA CTG 23

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GAATTTGAGT GAGTTTTTGA AGATGTATC 29

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

GAGCTGGATG TTGAGAGTGG AGAT 24

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GAGTCACTGC TGCTGCTTAT GTC 23

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

GATGGCACTC TGGTCACTGT G 21

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GCCACGCCTT CCGCTAAC 18

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

GCCCAGCTCC TTCCTCAAGT 20

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

GCTGCTGACC AAAGAGGACT T 21

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

GCTTGAGAGG GAAGACAATG AG 22

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

GGATACCTTT GCCATCTGTG TC 22

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

GGCAAGGATT TGGTGTGAGA T 21

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

GGGCGTCAAC AACCTCACTG 20

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

GGGTGACTGG CAGCACAGAT 20

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

GGTGGCGGTT ATGGCAATC 19

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

GTCTGCCGTC TCCACTTTGT C 21

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

GTGCGCCAGG TGGTAGCTC 19

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

GTTCATGTTG GGTTTGCTCT TC 22

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

TCTCCTATCT CGGGTGAAAT GTC 23

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

TGCCAATGTT AAGAAAGCAG ATAG 24

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

TGCCGTGTTA GGTTTGCAGA C 21

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

TGGACATTAT CGGCACCATT AC 22

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

TTCGGCTGCC TCCTCTATTT AC 22

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

TTCTCGTCCA GCCCTTCTAC C 21

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

TTGCCCTCTG ACCCTCTAGT CT 22

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

TTTAGAGGGG AAAACACAGA TGG 23

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

TTTGAAGGCT CCCATGATTC TG 22

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

TTTTCCTCTT CTCGCCGTTT CA 22

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

TTTTGGTTTG GGCTTCACAC 20

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

ACACCATTCC CCATTGTGAT TAT 23

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

ACTGCCCGGT TGTCGTGTC 19

(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

AGACCGGCCC CTCTGAATAG 20

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

AGCACGGAGC AGAGGAAGTT G 21

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

AGCAGATGGA GTCCACAGGA TCAG 24

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

AGCAGCACCA CCAAGAAGAA T 21

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

AGGTTCCGCT CTCGCACTT 19

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

CACGTTCCTG ATCTCCTCTG AC 22

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

CAGCGCGACT ACGAGGAGAT 20

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

CAGCGGTGGC TATGGACAG 19

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

CATGGGGTCC ACGTAGATGT AC 22

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

CATGTTGTCC AGCCGCATCA G 21

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

CCCATAGTGG TAGCCTGAGG AC 22

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

CCCCAGGCAT ATTTGACTCT C 21

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

CCCGCTCCTA CCCTGCAAAC 20

(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

CCTCATTCAG GTGATGTGCT CTAT 24

(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

CGCCTTGCCC AGTACTTGTC 20

(2) INFORMATION FOR SEQ ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

CGTCGAATCA AGACCTGCTT C 21

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

CGTTGTCGGT GTAAATGAAC TG 22

(2) INFORMATION FOR SEQ ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

CTACGACGGG GGTCTCCAC 19

(2) INFORMATION FOR SEQ ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

CTCATCGGGA AGACCTGGCT TAC 23

(2) INFORMATION FOR SEQ ID NO: 105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

CTCCATTTCA GAGTCATTGT CGTTAT 26

(2) INFORMATION FOR SEQ ID NO: 106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

CTTCCCCTGG ATGGAGAGTA AC 22

(2) INFORMATION FOR SEQ ID NO: 107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

CTTGGGTCGT TGGGCATTC 19

(2) INFORMATION FOR SEQ ID NO: 108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

GAGCAAAGAT CAAAATCAAA TGTT 24

(2) INFORMATION FOR SEQ ID NO: 109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

GAGGACAGCA TTCGCATATC AG 22

(2) INFORMATION FOR SEQ ID NO: 110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

GAGGTTTTCG AGGACTAGTT TTAACTGA 28

(2) INFORMATION FOR SEQ ID NO: 111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

GCCAGTGTAC GCCTTCTCCA T 21

(2) INFORMATION FOR SEQ ID NO: 112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

GCGATGCCCC AGCTCTAAC 19

(2) INFORMATION FOR SEQ ID NO: 113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

GCGCATCGGT CATTTTGAG 19

(2) INFORMATION FOR SEQ ID NO: 114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

GGACCGCCAA GAAAAGAAGT 20

(2) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

GGCAAACTGA GCGCATGTTA C 21

(2) INFORMATION FOR SEQ ID NO: 116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

GGTGCCTTCC CAGGTGATG 19

(2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

GGTTCAGGGC CAGTGCATAT T 21

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

GGTTTCTTCT TGGGGGCTTT AACT 24

(2) INFORMATION FOR SEQ ID NO: 119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

GTAGAGCCAG CCAGAGAAAA CAC 23

(2) INFORMATION FOR SEQ ID NO: 120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

GTCTCTCGCT CTGGCACAAG 20

(2) INFORMATION FOR SEQ ID NO: 121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

GTGGGCATGA AGTCAGAGAG C 21

(2) INFORMATION FOR SEQ ID NO: 122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

GTTTTTGGTT TTGGGTTACA GAACT 25

(2) INFORMATION FOR SEQ ID NO: 123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

GAACATAGAG GGCACTGACT GTAAG 25

(2) INFORMATION FOR SEQ ID NO: 124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

TCCTCGTCCA GCTGGTCTTG 20

(2) INFORMATION FOR SEQ ID NO: 125:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

TGAGCGCCTG CATGTTGAC 19

(2) INFORMATION FOR SEQ ID NO: 126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

TGAGGAACCA GAGAGCTTCT TTAC 24

(2) INFORMATION FOR SEQ ID NO: 127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

TGCTGGCAAT GGGAGCTCTC 20

(2) INFORMATION FOR SEQ ID NO: 128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

TGGCTGGCAA TGATGAAAAC 20

(2) INFORMATION FOR SEQ ID NO: 129:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:

TGGGCTGTCT GGAGTTTGAT G 21

(2) INFORMATION FOR SEQ ID NO: 130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

TGTCGGCTAA ATCCCAAATC T 21

(2) INFORMATION FOR SEQ ID NO: 131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

TTCCACTAGA GGTGTGTGCA GAG 23

(2) INFORMATION FOR SEQ ID NO: 132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:

TTCCCCATGA CTGGAGACAT AC 22

(2) INFORMATION FOR SEQ ID NO: 133:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:

AAAGGGTTCA GAAAAACTTC TTATCATC 28

(2) INFORMATION FOR SEQ ID NO: 134:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA (synthetic)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

AACTGCTGTT GCCTGGTTGA T 21

(2) INFORMATION FOR SEQ ID NO: 135:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

CTGGCAGCAC AGATGGTTTC 20

(2) INFORMATION FOR SEQ ID NO: 136:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

AATTTCGGTC AGAGCCACTT CTA 23

(2) INFORMATION FOR SEQ ID NO: 137:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:

ATGGGTGGAG CAAGAGGTTC 20

(2) INFORMATION FOR SEQ ID NO: 138:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:

ATCTACTCCC CGGATCACTC A 21

(2) INFORMATION FOR SEQ ID NO: 139:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:

AGGACCGCCA AGAAAAGAAG 20

(2) INFORMATION FOR SEQ ID NO: 140:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:

AGGGTTCAGC ATCCACCAAG 20

(2) INFORMATION FOR SEQ ID NO: 141:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:

CTTCTAGGTC TCCCACGAGG TT 22

(2) INFORMATION FOR SEQ ID NO: 142:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

GGGCATGTCA TCAGGAAACA C 21

(2) INFORMATION FOR SEQ ID NO: 143:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:

CAGCAGATGG AGTCCACAGG AT 22

(2) INFORMATION FOR SEQ ID NO: 144:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:

GTATTTTTCA TATACAGGAT TCCCACT 27

(2) INFORMATION FOR SEQ ID NO: 145:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:

GTGGGTAGAA GGGAGGCTAA AG 22

(2) INFORMATION FOR SEQ ID NO: 146:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:

TTCCACTAGA GGTGTGTGCA GA 22

(2) INFORMATION FOR SEQ ID NO: 147:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:

CGACCCCAAC GTCCCAGA 18

(2) INFORMATION FOR SEQ ID NO: 148:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

CGGTCATCCT GGGGCATATT T 21

(2) INFORMATION FOR SEQ ID NO: 149:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

TCAGCCGAGT AGTTTTCATC ATT 23

(2) INFORMATION FOR SEQ ID NO: 150:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

CATGTTGTCC AGCCGCATC 19

(2) INFORMATION FOR SEQ ID NO: 151:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

GGCCAGCACC TCCACCAT 18

(2) INFORMATION FOR SEQ ID NO: 152:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

TCGATCTTCC TTTTGGTCCA TATT 24

(2) INFORMATION FOR SEQ ID NO: 153:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

GCTGTAGGAG AATGGCTCGT G 21

(2) INFORMATION FOR SEQ ID NO: 154:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

CCTCCTCTTC TTCGTCCTGG TT 22

(2) INFORMATION FOR SEQ ID NO: 155:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:

TACTGCATGG AAACTTTTGG TG 22

(2) INFORMATION FOR SEQ ID NO: 156:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 156:

GTGGCTATGG ACAGCAGGAC 20

(2) INFORMATION FOR SEQ ID NO: 157:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:

CCCAGCTTTT CCGTTCACTT 20

(2) INFORMATION FOR SEQ ID NO: 158:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:

TCACAGTCTT CGCAGCGATA TT 22

(2) INFORMATION FOR SEQ ID NO: 159:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:

CACTGTCACT GTCCTCACTG TCACT 25

(2) INFORMATION FOR SEQ ID NO: 160:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:

TTCATCAGCA CCACCAACAC 20

(2) INFORMATION FOR SEQ ID NO: 161:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161:

CCTCCGTGGT GGGCTTCTT 19

(2) INFORMATION FOR SEQ ID NO: 162:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:

GCTGTTGTCA CTCTCGCTGT C 21

(2) INFORMATION FOR SEQ ID NO: 163:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 163:

AGGACCGCCA AGAAAAGAAG 20

(2) INFORMATION FOR SEQ ID NO: 164:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 164:

AGCAGATGGA GTCCACAGGA TCA 23

(2) INFORMATION FOR SEQ ID NO: 165:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 165:

GCCTCCTCGC CTGACGAAG 19

(2) INFORMATION FOR SEQ ID NO: 166:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 166:

AGCAGGAACG AATTTCATAT ACAC 24

(2) INFORMATION FOR SEQ ID NO: 167:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 167:

CAATCACCTT CCATCGGATC TC 22

(2) INFORMATION FOR SEQ ID NO: 168:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 168:

AAAAAACTCA TCCACTCAGC AATAG 25

(2) INFORMATION FOR SEQ ID NO: 169:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:

GACGCCACTT CATGTTCCA 19

(2) INFORMATION FOR SEQ ID NO: 170:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:

GCCAAAGCAT GATGAGGATG ATA 23

(2) INFORMATION FOR SEQ ID NO: 171:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:

AGCCACGAAT GTCCCAAATC T 21

(2) INFORMATION FOR SEQ ID NO: 172:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 172:

GGAATGGCAA GGAAGGCTAA 20

(2) INFORMATION FOR SEQ ID NO: 173:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 173:

TTGGGCAAGG ATTTGGTGT 19

(2) INFORMATION FOR SEQ ID NO: 174:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 174:

CACCGAAATC AAATGGAAAT CT 22

(2) INFORMATION FOR SEQ ID NO: 175:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 175:

TGAAGGCTCC CATGATTCTG 20

(2) INFORMATION FOR SEQ ID NO: 176:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 176:

GCTGGTCTTG CAGGCTGTTC 20

(2) INFORMATION FOR SEQ ID NO: 177:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 177:

GCAGCTTCGT AGACACGTTG A 21

(2) INFORMATION FOR SEQ ID NO: 178:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 178:

GCCTGACACC TT 12

(2) INFORMATION FOR SEQ ID NO: 179:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 179:

CTGCCCACAC C 11

(2) INFORMATION FOR SEQ ID NO: 180:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 180:

GCCACTAAGC AG 12

(2) INFORMATION FOR SEQ ID NO: 181:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 181:

TAATCCTCGT CTT 13

(2) INFORMATION FOR SEQ ID NO: 182:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 182:

GTCCTCTTCA ACC 13

CLAIMS

1. A method for detection of the presence or absence of chromosomal abnormalities, each chromosomal abnormality being associated with a condition in a subject and each chromosomal abnormality being defined by at least one characteristic nucleic acid sequence, the method comprising

a) obtaining a sample of nucleic acids derived from a subject which may harbour one of said chromosomal abnormalities,

b) subjecting the sample of nucleic acids to a multiplex molecular amplification procedure, wherein a number of said characteristic nucleic acid sequences, if present in a sufficient amount, will be amplified,

c) retrieving the product(s) from step b), and detecting the presence and/or absence of amplified characteristic nucleic acid sequences and thereby the presence or absence of corresponding chromosomal abnormalities,

wherein the multiplex molecular amplification procedure comprises the use of at least 7 mutually distinct primers in one single reaction mixture, each of the at least 7 mutually distinct primers defining an end of at least one characteristic nucleic acid sequence, and wherein at least one of the at least 7 mutually distinct primers defines the first ends of at least two characteristic nucleic acid sequences, said at least two characteristic nucleic acid sequences each being defined in their opposite ends by mutually distinct primers selected from the remainder of the at least 7 mutually distinct primers, whereby the number of amplified characteristic nucleic acid sequences which can be detected upon conclusion of the amplification reaction is at least ½×n+1, wherein n is the number of the at least 7 mutually distinct primers.

2. A method for detection of the presence or absence of chromosomal abnormalities, each chromosomal abnormality being associated with a condition in a subject and each chromosomal abnormality being defined by at least one characteristic nucleic acid sequence, the method comprising

a) obtaining a sample of nucleic acids derived from a subject which may harbour one of said chromosomal abnormalities,

b) subjecting the sample of nucleic acids to a multiplex molecular amplification procedure, wherein a number of said characteristic nucleic acid sequences, if present in a sufficient amount, will be amplified,

c) retrieving the product(s) from step b), and detecting the presence and/or absence of amplified characteristic nucleic acid sequences and thereby the presence or absence of corresponding chromosomal abnormalities,

wherein the multiplex molecular amplification reaction comprises

1) the use of an internal positive standard containing I) a nucleic acid fragment present in the sample, and II) primers for amplification of a nucleotide sequence of said nucleic acid fragment, and

2) a number, n, of mutually distinct primers each defining an end of a characteristic nucleic acid sequence,

and wherein at least one of the n mutually distinct primers defines first ends of at least two mutually distinct characteristic nucleic acid sequences, said at least two mutually distinct characteristic nucleic acid sequences being defined in their opposite ends by at least two mutually distinct primers selected from the remainder of the n mutually distinct primers, whereby the number of amplified characteristic

nucleic acid sequences which can be detected upon conclusion of the amplification procedure is at least ½×n+1.

3. A method according to claim 1 or 2, wherein n is 7.

4. A method according to claim 1 or 2, wherein n is 8.

5. A method according to claim 1 or 2, wherein n is 9.

6. A method according to claim 1 or 2, wherein n is 10.

7. A method according to claim 1 or 2, wherein n is 11.

8. A method according to claim 1 or 2, wherein n is 12.

9. A method according to claim 1 or 2, wherein n is 13.

10. A method according to claim 1 or 2, wherein n is 14.

11. A method according to claim 1 or 2, wherein n is 15.

12. A method according to claim 1 or 2, wherein n is 16.

13. A method according to claim 1 or 2, wherein n is 17.

14. A method according to claim 1 or 2, wherein n is 18.

15. A method according to claim 1 or 2, wherein n is 19.

16. A method according to claim 1 or 2, wherein n is 20.

17. A method according to claim 1 or 2, wherein n is 21.

18. A method according to claim 1 or 2, wherein n is 22.

19. A method according to claim 1 or 2, wherein n is 23.

20. A method according to claim 1 or 2, wherein n is 24.

21. A method according to claim 1 or 2, wherein n is 25.

22. A method according to claim 1 or 2, wherein n is in the range of 30 to 34.

23. A method according to claim 1 or 2, wherein n is in the range of 35 to 39.

24. A method according to claim 1 or 2, wherein n is in the range of 40 to 44.

25. A method according to claim 1 or 2, wherein n is in the range of 45 to 50.

26. A method according to any of the preceding claims, wherein the sample of nucleic acids derived from the subject is in the form of cDNA.

27. A method according to claim 26, wherein the cDNA is obtained by use of specific or non-specific cDNA primers in a molecular amplification procedure wherein the templates in the procedure are in the form of mRNA derived from the subject.

28. A method according to claim 27, wherein the cDNA primers are specific.

29. A method according to claim 28, wherein the number of cDNA primers is at least 20.

30. A method according to claim 28, wherein the number of cDNA primers is at least 50.

31. A method according to claim 28, wherein the number of cDNA primers is at least 100.

32. A method according to claim 28, wherein the number of cDNA primers is at least 150.

33. A method according to claim 28, wherein the number of cDNA primers is at least 200.

34. A method according to any of claims 26-33, wherein the conditions for obtaining cDNA derived from the subject are compatible with the conditions of the molecular amplification procedure.

35. A method according to any of the preceding claims, wherein said multiplex molecular amplification is a multiplex polymerase chain reaction.

36. A method according to claim 8, wherein said multiplex polymerase chain reaction is a nested polymerase chain reaction.

37. A method according to any of the preceding claims, wherein the chromosomal abnormality is the presence of a transcribed fusion gene.

38. A method according to claim 29, wherein the presence of the transcribed fusion gene is the result of an inversion.

39. A method according to claim 29, wherein the presence of the transcribed fusion gene is the result of a deletion.

40. A method according to claim 29, wherein the presence of the transcribed fusion gene is the result of a duplication.

41. A method according to claim 29, wherein the presence of the transcribed fusion gene is the result of activation of a proto-oncogene, such as Hox-11 and evi-1.

42. A method according to any of the preceding claims, wherein at least one chromosomal abnormality is associated with a malignant neoplastic condition.

43. A method according to claim 42, wherein the malignant neoplastic condition is a systemic neoplastic malignancy.

44. A method according to claim 43, wherein the systemic neoplastic malignancy is selected from the group consisting of leukaemia such as acute leukemia (AL), chronic leukemia (CL), T-cell acute leukemia (T-ALL), B-cell acute leukemia (B-ALL), T-cell chronic leukemia (T-CLL), B-cell chronic leukemia (B-CLL), prolymphocytic leukemia (PLL), acute undifferentiated leukemia (AUL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), acute promyelocytic leukemia (APL), pre-B-ALL, and pro-B-ALL;
lymphoma such as Burkitt's lymphoma (BL), non-Hodgkins lymphoma (NHL), Hodgkins lymphoma (HL), follicular lymphoma (FL), diffuse large cell lymphome (DLCL), T-cell lymphoma, B-cell lymphoma;
myelodysplasia; and
myeloid.

45. A method according to claim 43 or 44, wherein the chromosomal abnormality is selected from the group consisting of:
dup(11q23) (dup exon 5-9/2);
dup(11q23) (dup exon 5-9/4);
inv(16)(p13;q22);
t(1;11)(p32;q23);
t(1;19)(q23;p13);
t(10;11)(p14;q23);
t(10;11)(p14;q23);
t(10;14)(q24;q11);
t(11;17)(q23;q21);
t(11;19)(q23;p13.1);
t(11;19)(q23;p13.3);
t(12;21)(p13;q22);
t(12;22)(p13;q11);
t(15;17)(q21;q22);
t(15;17)(q21;q22);

t(16;21)(p11;q22);
t(17;19)(q22;p13);
t(2;3)(p21;q26);
t(2;5)(p23;q35);
t(3;21)(q26;q22);
t(3;3)(q21;q26);
t(3;5)(q25.1;q34);
t(4;11)(q21;q23);
t(5;12)(q33;p13);
t(5;17)(q35;q22)
t(6;11)(q27;q23);
t(6;9)(p23;q34);
t(7;10)(q35;q24);
t(7;9)(q34;q32);
t(8;21)(q22;q22);
t(9;11)(q22;q23);
t(9;12)(q34;p13);
t(9;22)(q34;q11)
t(9;22)(q34;q11)
t(X;11)(q13;q23); and
$tal1^{d1-3}$ (40 kb deletion), or wherein the chromosomal abnormality is selected from the genes in the group CBFβ/MYH11, SIL1/TAL1, MLL1, EVI-1, MLL1/AFX1, MLL1/AF1p, MLL1/AF1q, E2A/PBX1, E2A/HLF, EVI1, NPM/ALK, NPM/MLF, AML1/EVI1, MLL1/-AF4, TEL/PDGfβ, NPM/RARα, DEK/CAN, SET/CAN, MLL1/AF6, HOX11, AML1/MTG8, MLL1/AF9, BCR/ABL, MLL1/AF10, MLL1/AF17, PLZF/-RARα, MLL/ELL, MLL/ENL, TEL/AML 1, PML/RARα, FUS/ERG, AML1/-MDS, AML1/EAP, TEL/MN1, MLL exon 5-9/2, and MLL exon 5-9/4.

46. A method according to claim 42, wherein the malignant neoplastic condition is a non-systemic neoplastic malignancy.

47. A method according to claim 46, wherein the non-systemic neoplastic malignancy is selected from the group consisting of carcinoma, adenocarcinoma, liposarcoma, fibrosarcoma, chondrosarcoma, osteosarcoma, leiomyosarcoma, rhabdomyosarcoma, glioma, neuroblastoma, medullablastoma, malignant melanoma, neurofibrosarcoma, heamangiosarcoma, lymphangiosarcoma,

malignant teratoma, dysgerminoma, seminoma, and choriocarcinoma.

48. A method according to claim 47, wherein the carcinoma is selected from carcinoma of the breast, bronchus, colorectum, stomach, prostate, ovary, lymphoid tissue, lymphoid marrow, uterus, pancreas, esophagus, urinary bladder, kidney, or skin.

49. A method according to claim 47, wherein the malignant neoplastic condition is selected from the group consisting of papillary thyroid carcinoma, Ewing's sarcoma, liposarcoma, rhabdomyosarcoma, synovial sarcoma, and melanoma of soft parts.

50. A method according to any of the preceding claims, wherein the sample of nucleic acids is derived from cells of the bone marrow in the subject.

51. A method according to any of claims 1-49, wherein the sample of nucleic acids is derived from peripheral blood cells in the subject.

52. A method according to any of claims 1-49, wherein the sample of nucleic acids is derived from placental cells.

53. A method according to any of claims 1-49 wherein the sample of nucleic acids is derived from foetal cells.

54. A method according to claim 1-49, wherein the sample of nucleic acids is derived from amniotic fluid.

55. A method according to any of the preceding claims, wherein at least one of the primers used in the multiplex molecular amplification procedure is labelled.

56. A method according to claim 55, wherein the label is selected from the group consisting of a radioactive label, a

coloured label, a fluorescent label, a biotinyl label, a phosphate label, an amin label, and a tiol label.

57. A method according to any of the preceding claims, wherein the sample of nucleic acids is subjected to at least two multiplex molecular amplification procedures as defined in any of the preceding claims.

58. A method according to claim 57, wherein the at least two multiplex molecular amplification procedures are carried out in parallel.

59. A method according to claim 57 or 58, wherein the at least two multiplex molecular amplification procedures are carried out under substantially the same conditions with respect to physical parameters and timing.

60. A method according to any of the preceding claims, wherein the presence or absence of the characteristic sequences is determined by means of gel electrophoresis, sequence analysis, HPLC, FPLC and by flouresence spectofotometri.

61. A method according to any of claims 2-60, wherein the nucleic acid fragment of the internal standard is a cDNA molecule derived from the subject.

62. A method according to claim 61, wherein the cDNA molecule is obtained by use of specific or non-specific cDNA primers in a molecular amplification procedure wherein the templates in the procedure are in the form of mRNA derived from the subject.

63. A method according to claim 62, wherein the cDNA molecule is obtained in the molecular amplification procedure defined in claim 27.

64. A method according to claim 62, wherein the cDNA molecule corresponds to a constitutively expressed DNA fragment.

65. A method according to any of the preceding claims, wherein primers used in the molecular amplification procedure are so constructed, that

1) they hybridize to their respective target sequences at or below substantially the same temperature,

2) they are substantially specific for their respective target sequences,

3) they exhibit substantially no intramolecular hybridization,

4) they have a higher melting point in the 5'-end than in the 3'-end,

5) no two primers are, in the molecular amplification procedure, capable of together initiating and sustaining amplification of nucleic acid fragments in the sample which correspond to normally occurring sequences not associated with a condition in the subject,

6) no primer contains more than 5 consecutive guanidyl residues,

7) they exhibit substantially no intermolecular hybridization.

66. A method according to claim 65 wherein the primers hybridize to their respective target sequences at a temperature difference within 5°C.

67. A method according to any of claims 65 and 66 wherein the primers are complementary to their respective sequence.

68. A method according to any of claims 65-67 wherein the primers have a delta G being above -1 within the primer.

69. A method according to any of claims 65-68 wherein the primers have a higher melting point in the 5'-end than in the 3'-end of at least 1°C, preferable at least 6°C.

70. A method according to any of claims 65-69 wherein no primer contains more than 3 consecutive guanidyl residues.

71. A method according to any of claims 65-70 wherein the primer dimer has a delta G being above -10.

72. A method according to any of the preceding claims, wherein the primers used in the molecular amplification procedure have nucleic acid sequences which are selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 182.

73. A method according to any of the claims 27-72, wherein the cDNA primers are selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 32 and SEQ ID NO: 178 through SEQ ID NO: 182.

74. A method according to any of the preceding claims wherein the sample material is 1 μg nucleic acid for each multiplex molecular amplication procedure.

75. A nucleic acid fragment which has a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 182.

76. A kit comprising 7 mutually distinct primers selected from the group of cDNA primers consisting of SEQ ID NO: 1 through SEQ ID NO: 32 and SEQ ID NO: 178 through SEQ ID NO: 182; and of PCR primers selected from SEQ ID NO: 33 trough SEQ ID NO: 177.

77. A kit according to claim 77 wherein the primers are attached to a device selected from the group comprising a well, a capillary tube, a stick, and a bead.

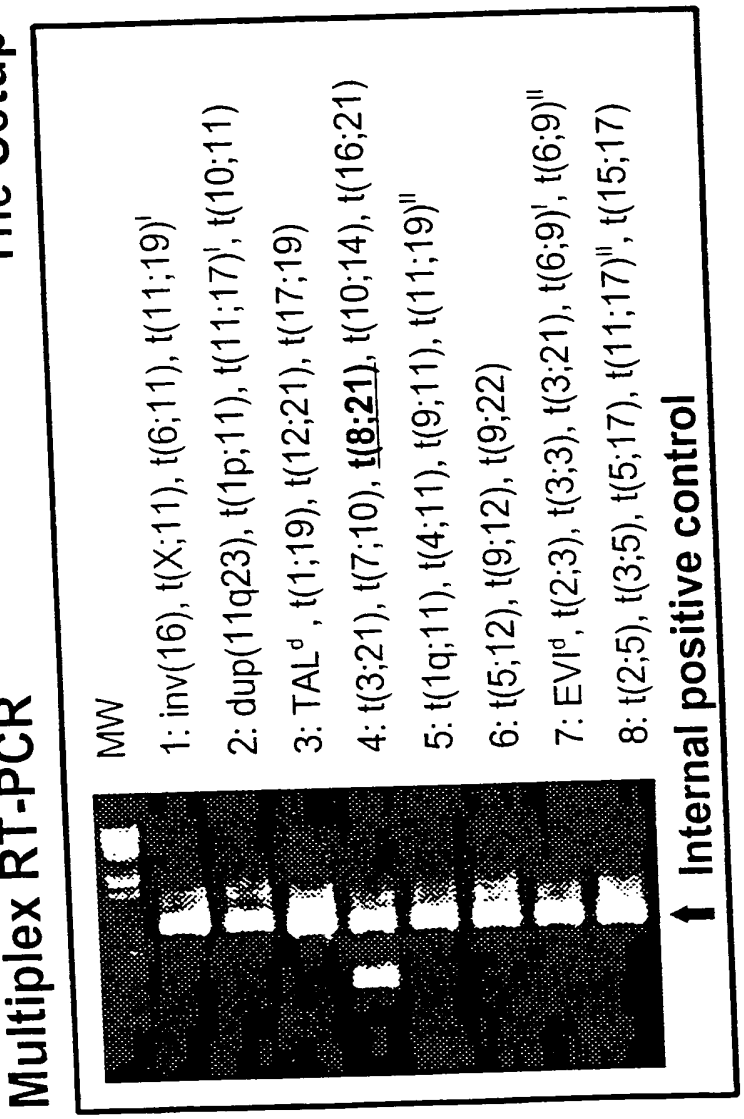
Multiplex RT-PCR
The Setup
MW
1: inv(16), t(X;11), t(6;11), t(11;19)'
2: dup(11q23), t(1p;11), t(11;17)', t(10;11)
3: TAL^{d}, t(1;19), t(12;21), t(17;19)
4: t(3;21), t(7;10), t(8;21), t(10;14), t(16;21)
5: t(1q;11), t(4;11), t(9;11), t(11;19)"
6: t(5;12), t(9;12), t(9;22)
7: EVI^{d}, t(2;3), t(3;3), t(3;21), t(6;9)', t(6;9)"
8: t(2;5), t(3;5), t(5;17), t(11;17)", t(15;17)
Internal positive control

Fig. 1

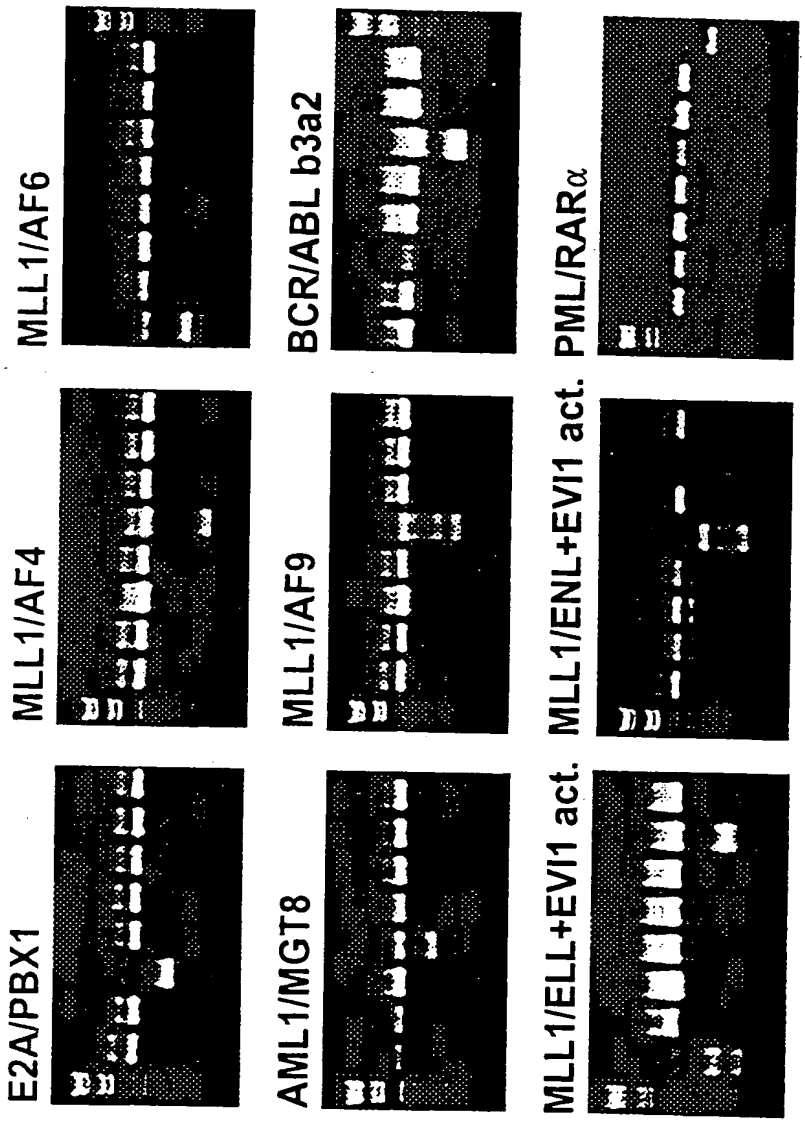

Fig. 2A

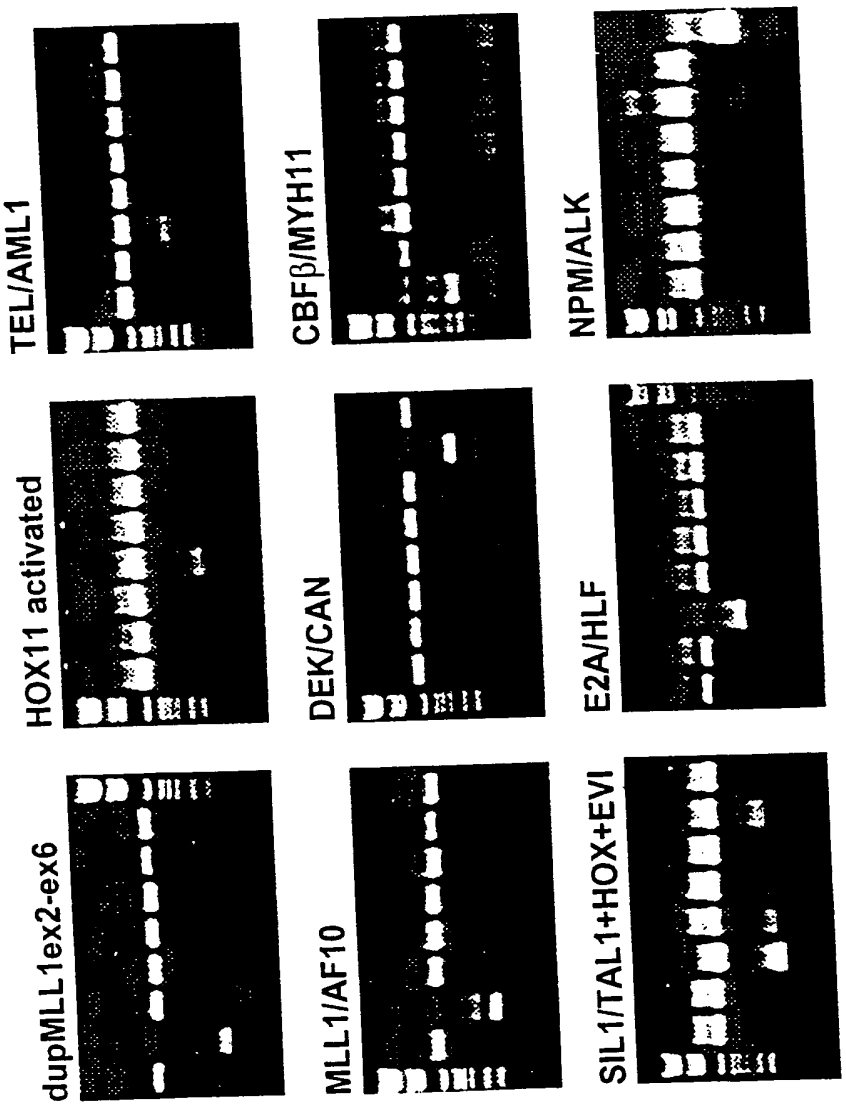
TEL/AML1
CBFβ/MYH11
NPM/ALK
HOX11 activated
DEK/CAN
E2A/HLF
dupMLL1ex2-ex6
MLL1/AF10
SIL1/TAL1+HOX+EVI

Fig. 2B

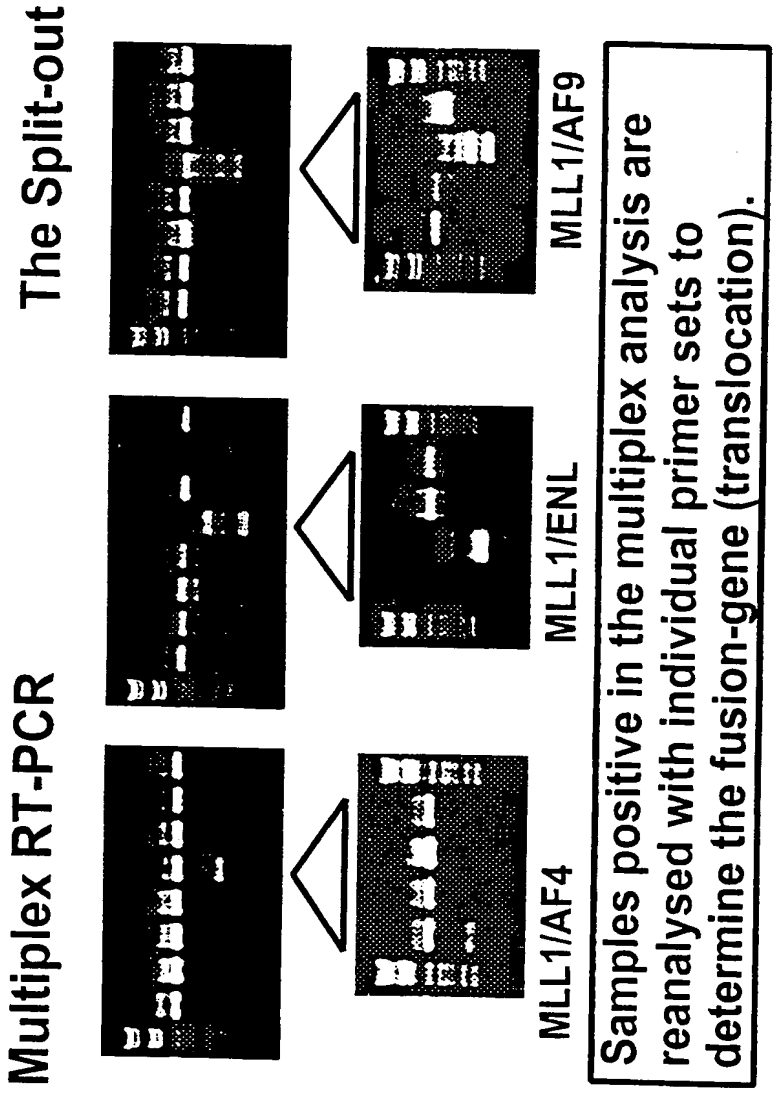

Fig. 3

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(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: HOKLAND, Peter [DK/DK]; Rouloen 18, DK–8250 Egå (DK).

(74) Agent: PLOUGMANN, VINGTOFT & PARTNERS A/S; Sankt Annæ Plads 11, P.O. Box 3007, DK–1021 Copenhagen K (DK).

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(54) Title: DETECTION OF CHROMOSOMAL ABNORMALITIES

(57) Abstract

The present invention has provided a method for detection of the presence or absence of chromosomal abnormalities which are associated with a condition e.g. leukaemia in a subject and are each defined by at least one characteristic nucleic acid sequence. In general, the method comprises subjecting a sample of nucleic acids to a multiplex molecular amplification procedure. The multiplex molecular amplification procedure comprises the use of at least 7 mutually distinct primers in one single reaction mixture, each of the at least 7 mutually distinct primers defining an end of at least one characteristic nucleic acid sequence, and wherein at least one of the at least 7 mutually distinct primers defines the first ends of at least two characteristic nucleic acid sequences, said at least two characteristic nucleic acid sequences each being defined in their opposite ends by mutually distinct primers selected from the remainder of the at least 7 mutually distinct primers, whereby the number of amplified characteristic nucleic acid sequences which can be detected upon conclusion of the amplification reaction is at least 1/2xn+1, wherein n is the number of the at least 7 mutually distinct primers. In one embodiment, the use of an internal positive standard containing: I) a nucleic acid fragment present in the sample, and II) primers for amplification of a nucleotide sequence of said nucleic acid fragment is incorporated into the procedure.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 97/00556

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12Q 1/68 // C12N 15/11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12Q, C12N

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Leukemia, Volume 9, 1995, Reinald Repp et al, "Detection of Four Different 11q23 Chromosomal Abnormalities by Multiplex-PCR and Fluorescence-Based Automatic DNA-Fragment Analysis", page 210 - page 215, see abstract and page 214, line 8 - line 16	1-77
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X	EP 0181635 A2 (ONCOGENE SCIENCE, INC.), 21 May 1986 (21.05.86), claim 16, fig. 3	75-77

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 December 1998	04 FEB 1999
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Authorized officer PATRICK ANDERSSON

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X	WO 9426930 A1 (THOMAS JEFFERSON UNIVERSITY), 24 November 1994 (24.11.94), claim 2, pages 99-106, claim 8, pages 116-118, claim 47, pages 133-134	75-77
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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-77
Inventions 1,4,7,10,11; see extra sheet

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

According to PCT rule 13.2, an international application shall relate to one invention only or a group of inventions linked by one or more of the same or corresponding "special technical features", i.e. features that define a contribution which each of the inventions considered as a whole makes over the prior art.

The claimed invention relates to a method for detecting chromosomal abnormalities. In the method at least 7 mutually distinct primers, each characteristic for a chromosomal abnormality, e.g. a translocation, are used. The application further claims nucleic acids and a kit using the nucleic acids. A unifying special technical feature linking the method with the nucleic acids would be that the nucleic acids are characteristic for chromosomal abnormalities, however such a link has not been contemplated in the application. Moreover nucleic acids characteristic for chromosomal abnormalities are well-known in the art, see e.g. Repp et al., in the search report.

The application fails, *a priori*, to comply with PCT-rule 13.2. No unifying special technical feature could be found. In principle the application contains 183 inventions. However, for the purpose of searching the inventions are randomly grouped as follows:

Invention 1, claims 1-74: a method for detecting chromosomal abnormalities

Invention 2, claims 75-77, partially: SEQ ID NO: 1-16 and related kit.

Invention 3, claims 75-77, partially: SEQ ID NO: 17-32 and related kit.

Invention 4, claims 75-77, partially: SEQ ID NO: 33-48 and related kit.

Invention 5, claims 75-77, partially: SEQ ID NO: 49-64 and related kit.

Invention 6, claims 75-77, partially: SEQ ID NO: 65-80 and related kit.

Invention 7, claims 75-77, partially: SEQ ID NO: 81-96 and related kit.

Invention 8, claims 75-77, partially: SEQ ID NO: 97-112 and related kit.

Invention 9, claims 75-77, partially: SEQ ID NO: 113-128 and related kit.

Invention 10, claims 75-77, partially: SEQ ID NO: 129-144 and related kit.

Invention 11, claims 75-77, partially: SEQ ID NO: 145-160 and related kit.

Invention 12, claims 75-77, partially: SEQ ID NO: 161-176 and related kit.

Invention 13, claims 75-77, partially: SEQ ID NO: 178-182 and related kit.

The search has been limited to inventions 1, 4, 7, 10 and 11.

SA 178798

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/12/98

International application No.
PCT/DK 97/00556

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